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The American Journal of Medical Technology

MARCH-APRIL, 1953

Vol. 19, No. 2

OFFICIAL PUBLICATION
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AMERICAN SOCIETY OF MEDICAL TECHNOLOGISTS
Published Bi-Monthly by The American Society of Medical Technologists
Printed by The Gulf Publishing Company
Business and Editorial Office: Suite 25, Hermann Professional Bldg., Houston 25, Texas
Printing Office: 3301 Buffalo Drive, Houston 6, Texas

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The American Journal of Medical Technology is owned by The American Society of Medical Technologists. It is published bi-monthly. The volume begins with the January issue.

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The
AMERICAN JOURNAL
of
MEDICAL TECHNOLOGY

VOLUME 19 MARCH-APRIL, 1953 NUMBER 2

DETECTING THE Du FACTOR

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In 1900, Landsteiner discovered that the human erythrocyte contained two properties which he called A and B agglutinogens. Thirty-seven years later through the work of Landsteiner and Wiener[3] and Levine and Stetson,[5] it was reported that the human erythrocyte contained another factor which was termed Rh. Today we know that the erythrocyte contains not only the agglutinogens of the A and B systems, Rh and hr, M and N, P, but also the Kell,[9] Lewis,[6] Lutheran,[1] S,[11] Kidd,[8] Cellano,[4] and Duffy[2] factors. It has been proven that an individual may be immunized against any of these factors which he does not possess. Immunization to the M, N and the rare Rh subgroups Cw[1] and Cv[7] seldom take place and at the present time are of little clinical importance; however the weakly reacting Rh positive blood that was first described in 1944 by Wiener[13] who termed it "intermediate" and then in 1946 by Stratton[12] who called it Du, may be as antigenic as other Rh positive bloods.

Du blood is a hazard that is not well recognized because generally it is typed as rh (cde/cde). Detecting the Du factor is of utmost importance since it is now apparent that in patients who are rh (cde/cde) and are given repeated blood transfusions may develop antibodies to this antigenic factor. In the conventional cross-matching techniques such antibodies are in many instances not detected. It was reported by Rosenfield, Vogel, Miller and Haber[12] that one blood in every 200 specimens which had been typed rh (cde/cde) had actually possessed the Du factor.

At the present time there is no anti-Rh serum which will detect all the weakly reacting Du bloods. Therefore the following technic should be applied to all bloods of donors typed as rh negative in blood banks.

A 2 to 5 percent suspension of the donor's cells should be prepared in normal saline. Two drops of the suspension are placed in a small test tube (10 x 75 mm.). To the two drops of the cell suspension one drop of anti-Rh_o (D) sera is added and then the tube is placed in a 37° C. water bath. At the end of the hour the tube is removed from the water bath and is centrifuged until the cells are sedimented. The supernatant fluid is removed from the cells and is replaced with a full tube of fresh saline. The saline is added in a forceful stream directly down into the center of the tube's contents so that the cells are resuspended. The tube is again centrifuged and the washing is repeated twice more. After the last washing the supernatant fluid is completely removed leaving the cells packed at the bottom of the tube. Two drops of an anti-human globulin serum are added to the cells and the tube is then placed in a 37° C. water bath for 15 minutes. Upon the removal of the tube from the water bath it is centrifuged at approximately 500 r.p.m. for 2 minutes. The tube is then examined for agglutination grossly by gently agitating. If there is no agglutination the blood can be classified as type rh (cde/cde), but if agglutination is present then the blood possesses the Du factor and must be classified as Rh positive.

It is important, although laborious, that all blood typed as rh negative be checked for the Du factor. In this way patients who are typed rh (cde/cde) and are receiving repeated transfusions of blood will be given Du negative blood and the chances of a hemolytic reaction occurring will be prevented.

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A MICRO-METHOD FOR PEDIATRIC SEROLOGIC TESTS

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It has been the experience at this hospital that the collection of blood specimens from children under one year is a time consuming and difficult procedure. Since immobilization of the arm and the assistance of a nurse is needed, much time may be consumed with each specimen. A more rapid and practical blood collecting procedure is important for the efficient scheduling of the technician's time. A method for quick collection of routine serologic tests has been developed at this hospital. This is accomplished by the use of minute amounts of capillary blood secured from a heel or finger puncture. The blood specimen is collected in a heparinized pipet. These pipets are made from 30 cms. of pyrex tubing with outside diameter of 3 mm. and inside diameter of 1.5 mm. by heating and drawing out central portion. The thin portion is cut with an ampule file making two collecting capillary pipets 11-15 mm. long. Heparin may be used as the anticoagulant with cardiolipin tests because it has little effect on the calcium in the blood clotting mechanism. Heparin does have an inhibitory action on the complement midpiece so this anticoagulant is not practical in complement fixation tests.

For the actual collection, the apparatus needed is the capillary pipet, 3 percent heparin, sealing wax and an alcohol lamp. The pipet is rinsed with 3 percent solution of heparin. The finger or heel is wiped with a germicide and the skin is punctured with a Bard-Parker blade. A few seconds wait at this time will give a better flow of blood from the puncture. The tip of the horizontally held capillary pipet is placed in the large drop of blood which forms and the blood is allowed to run into the tube. Gentle pressure on surrounding area will supply enough blood to fill tube. The pipet is sealed on one end with Dekonisky's or red sealing wax and the blood is shaken down in the tube by a snap of the wrist. This prevents clotting in the tip. The collecting pipets are placed in labeled 13 x 100 mm. culture tube and

centrifuged for 10 minutes at 1500 rpm. The capillary tube is broken ¼ inch above packed cells and resealed with wax. This portion and its contents are inactivated for 5 minutes at 60° by placing in a Kahn serologic tube filled with water from water bath.

The VDRL cardiolipin slide flocculation serologic test is used on these micro-specimens. Approximately 0.05 ml. of inactivated serum is placed in numbered depressions of a Rein slide. A drop of properly mixed antigen from a No. 23 or No. 24 gauge needle is placed upon the serum and a standard positive and negative control. The slides are rotated for 4 minutes on a rotator. The slide is then removed and examined under low power of microscope. The various serums are reported as positive, doubtful, and negative; according to the degree of flocculation.

An evaluation run on parallel control specimens of venipuncture clotted blood and those collected in heparinized capillary pipets showed no conflicting results.

This practical method of collection and the adaptation to the VDRL cardiolipin slide test has proven very successful and accurate in over 300 individual routine serologic tests on infants and small children. As with any screening test, doubtful or positive results should have further study by complement fixation and other macrotechnic tests.

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DISODIUMETHYLENEDIAMINE-TETRA-ACETATE

An Anticoagulant for Routine Hematological Work*

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The anticoagulant properties of EDTA§ and its salts were first investigated by Dyckerhoff[1] and coworkers in 1942. They found that minimal quantities of these compounds prevented the coagulation of blood in vitro but not in vivo. They are ten times more effective than sodium citrate. The anticoagulant effect is due to the binding of the blood calcium ion.

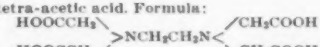
F. Proescher[2] re-investigated the disodium salt of EDTA and found that it is not only a potent anticoagulant but also a good preservative for the cellular elements of the blood and the plasma constituents.

The EDTA and its alkali salts are very powerful di-ionizing agents for alkali earth and heavy metals. They are non-colloidal organic, chelating or complexing agents, forming soluble compounds of high stability. These colorless substances are available as free acids and the di-, tri-, and tetra sodium salts under the trade name Sequestrene.§§

The free acid is only slightly soluble in water, whereas the sodium salts readily dissolve in water. Neither the free acid nor its salts hydrolyze in aqueous solution. They are stable and effective over a wide range of temperature and pH. The disodium salt has a solubility of 10% in water at a pH 5. It can be added to blood either as a dry substance or as an aqueous solution. A 10% solution is most convenient to use. 0.05-0.1 cc will prevent 5 cc of blood from coagulating. This amount corresponds to a concentration of 1-2 mg. sequestrene per cc of blood.

Proescher found that the erythrocytes, leokocytes and platelets show practically no numerical change from 2-3 days. The stainability is well preserved. After three days the polymorphonuclear neutrophiles and eosinophiles show beginning degenerative changes of the nuclei. The granules, especially the eosinophilic and basophilic, remain stainable. Monocytes degenerate after 1-2 weeks. The lymphocytes are the most resistent and remain stable for 2-3 weeks. The peroxydase reaction of the granules remains positive after complete destruction of the

§Ethylenediamine-tetra-acetic acid. Formula:



§§Manufactured by ALROSE Chemical Company, Providence, R. I. A chemically identical compound is manufactured by BERSWORTH Chemical Company, Framingham, Mass., under the trade name VERSENE.

* Registry Award Paper, read before ASMT Convention, Portland, Oregon, June, 1952.

nuclei and the cytoplasm. Sedimentation rate remains constant for at least 3-5 days. Serum bilirubin remains unchanged if the blood is centrifuged before hemolysis occurs. Prothrombin time remains unchanged for several days.

Comparative Studies of SNA_2§ With Other Anticoagulants

In our laboratory a number of studies were made in order to compare the suitability of SNA_2 with other common anticoagulants. Ten blood specimens were drawn and each mixed with the following anticoagulants: powdered heparin, ammonium potassium oxalate, potassium oxalate and SNA_2.

1) Studies on hematocrits: Using the heparinized specimens as a standard (100%) we found that the hematocrits of the SNA_2 specimens showed values of 100% of the standard in 8 samples and 101% in two samples. The hematocrits of the ammonium-potassium oxalate specimens showed values between 100% and 105% of the standard. The greatest discrepancies were observed on the potassium oxalate specimens with values ranging from 87%-95.2%.

2) A study on sedimentation rates is in progress and will be reported at a later date.

3) Studies on hemolysis: Another series of blood samples were taken using potassium oxalate in the first and SNA_2 in the second tube. The tubes were stored at 5° C. Of the 12 different blood samples, two specimens taken with potassium oxalate showed slight initial hemolysis increasing gradually to marked after 5 days. The corresponding SNA_2 specimens remained unhemolyzed until the 5th day when very slight hemolysis could be observed. In these tubes marked hemolysis was first seen after 13 days. Six blood specimens taken with potassium oxalates showed slight hemolysis after one day, progressing to marked after 5-6 days. The corresponding SNA_2 specimens showed slight hemolysis after 8-11 days progressing in four tubes to marked after 13 days and remaining slight in the two other tubes after 15 days. Four specimens showed beginning hemolysis in the potassium oxalate tubes after two days, progressing to marked after 8 days. The hemolysis in the corresponding SNA_2 tubes started after 8 days with only little change after 15 days (see Table I).

Blood specimens taken with 4 different anticoagulants and stored at 5° C. showed the most hemolysis in ammonium potassium oxalate and potassium oxalate tubes, and the least hemolysis in SNA_2 and heparin tubes. The degree of hemolysis conformed with the one in the first series of studies.

§Trade name for the disodiumethylenediamine-tetra-acetate.

TABLE I
Degree of hemolysis in potassium oxalate and SNA_2 tubes.

Anticoagulant:	After 1 Day	After 3 Days	After 5 Days	After 6 Days	After 8 Days	After 10 Days	After 13 Days	After 15 Days
Potassium oxalate tubes No. 1, 2	+1	+3	+4	+4	+4	+4	+4	+4
SNA_2 tubes No. 1, 2	neg.	neg.	+1	+1	+2	+3	+4	+4
Potassium oxalate tubes No. 3-8	+1	+2	+3	+4	+4	+4	+4	+4
SNA_2 tubes No. 3-8	neg.	neg.	neg.	neg.	+1	+2	+4	+4
SNA_2 tubes No. 7, 8	neg.	neg.	neg.	neg.	+1	+1	+1	+1
Potassium oxalate tubes No. 9-12	neg.	+1	+1	+3	+4	+4	+4	+4
SNA_2 tubes No. 9-12	neg.	neg.	neg.	neg.	+1	+1	+1	+2

SYMBOLS:—neg.—negative
+1 very slight hemolysis
+2 slight hemolysis
+3 moderate hemolysis
+4 marked hemolysis

4) Studies on blood counts: Red and white counts taken from the potassium oxalate and SNA_2 specimens on the first day were in agreement with each other and fingertip blood counts. Smears made after 1, 2, and 3 days showed picnotic changes and slight destruction of granulocytes in the potassium oxalate specimens on the second day, progressing to a point after 3 days where most of the granulocytes appeared disintegrated. The corresponding SNA_2 specimens showed very little change after 24 hours and disintegration of about 30% of granulocytes after 3 days. Lymphocytes remained unchanged.

5) Agglutinogens for blood type and Rh factor remained unchanged in both specimens for a period of 15 days.

6) In the use of SNA_2 for blood chemistry special consideration should be given to the fact that Sequestrene contains 2 nitrogen atoms. Spot tests for blood sugar yielded identical results with potassium oxalate and SNA_2. More work is in progress in order to evaluate the advantage, if any, of this anticoagulant for chemistry tests.

Summary

A new anticoagulant is evaluated as to its usefulness in hematological work. It was shown that blood taken with this anticoagulant and kept at 4-5° C remained longer free of hemolysis and that the cellular elements were better preserved than with the usual anticoagulants. Hematocrits conformed closer with the standard heparin hematocrits than the ammonium-potassium oxalate blood specimens. These studies seem to indicate that Disodiumethylenediamine-tetra-acetate might be a better anti-

coagulant for hematological routine work than the anticoagulants now in use.

Acknowledgments

Grateful acknowledgment is made to Dr. Frederick Proescher, Director of the Santa Clara Blood Center, San Jose, California, for his help in preparing this paper and permission to use his data; and to Dr. Donald Alcott, Pathologist of the Santa Clara County Hospital, for his helpful suggestions in the course of the investigation.

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A REVIEW OF SOME BASIC PRINCIPLES AND TECHNIQUES IN HEMATOLOGY

MARGRET E. HUGHES, B.A., M.T. (ASCP)*

This report is designed to be a review of some basic principles useful in evaluating standard or new hematologic methods in the clinical laboratory, with brief notations pertaining to the relative merits of some techniques. In order to evaluate new laboratory procedures in any field one must have a thorough knowledge of the old method, to form a base-line for comparison. While all clinicians and laboratory personnel try always to be alert in recognizing a better way of obtaining dependable laboratory data, the quality of newness of a technique does not necessarily indicate that it is a better method. If there is an appreciation of the amount and the accuracy of information obtainable by the standard technique, then one is better equipped to judge the relative merits of a new substitute method or modification for obtaining this same kind of information. The primary considerations in evaluating new and old laboratory techniques, for the purpose of comparison, are a thorough understanding of the range of normal values for the particular method, the inherent errors of the method, the efficiency and relative ease of performance, and the degree of validity or the significance of the information obtained.

The Range of Normal

The mean or average normal values for the different age and sex groups, as well as the ranges of normal values, by the particular method that is being used (or evaluated) in a given laboratory should be determined. The range of normal and the mean normal value for a biologic constituent or function may vary greatly depending on the method used for its estimation, and this applies particularly to modifications of standard methods—

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i.e., the range of normal for a standard technique does not necessarily apply to all modifications of the technique, even though slight—; the range of normal is also not necessarily exactly the same in all laboratories using the same technique. Equally important, the known inherent error of a standard method is not necessarily the same for modifications of the method. As guides in assessing mean normal values and ranges of normal for various hematologic procedures the recent publication by the Committee on the Handbook of Biological Data[1] and the book by Sunderman and Boerner[2] are helpful. Each contains an extensive list of references, giving the authorities and techniques used.

The range of normal values resulting from a new or modified test should be determined in every laboratory by the approved procedure of accumulating statistically significant data on the range that results when the test is applied to at least twenty *healthy* persons; hospital or ambulatory clinic patients do not fall into this category and are unsuitable for establishing ranges of normal. A range of normal does not apply to a test whose result is either positive or negative, but all such procedures must be evaluated in the laboratory for their specificity to determine the relative number of false-positive or false-negative results that are likely to occur and, if possible, under what conditions and circumstances such false results most frequently are encountered.

The inherent error of the test itself can be best determined by performing a series of at least twenty successive estimations using the same specimen, these estimations being performed by the same person. Such a procedure is also valuable in ascertaining the proficiency of new personnel or of student technologists. The technique of an acceptable procedure must be sound enough so that reproducible results can be obtained by different persons. It is not sufficiently worth while if only one person using the technique can reproduce his own results. This requirement alone has proved many laboratory procedures to be worthless, or of extremely limited value. Biggs and MacMillan[3] have reported the results of experiments they devised to assess the errors inherent in the routine use of some common hematologic methods.

Because of biologic variations—normal variations between individuals and normal variations in the same individual from day to day, or even diurnally—and the recognized overlapping of the extremes of normal by the abnormal, a normal range that will include the normal occurrences of any phenomenon 100 per cent of the time is theoretically impossible and would be of little value. The range of normal, based on a large, representative sample of the normal population, that will include 95 per cent (plus or minus two standard deviations of the distribution) of the results that can be expected to occur in perfectly healthy persons is the most useful range. It should be pointed out that all

ranges of normal depend basically on standard, competent, and experienced manipulations, the more accurate results being obtained by means of special refinements, such as the use of Bureau of Standards-certified equipment and other highly accurate adjuncts, which minimize the inherent errors of methods, but which are usually not essential for most clinical purposes, so long as accurate, unmodified manipulations are assured.

In the laboratory one of the greatest contributors to inaccurate or erroneous results is the introduction, either unconsciously or intentionally, of personal modifications of techniques without determining the effect of such a modification on the range of normal values or the degree to which it contributes to the inherent error of the standard technique. Most modifications are adopted primarily for the purposes of simplification or of increasing speed and quantity of work, but these purposes alone do not justify themselves if by them accuracy and dependability are sacrificed. One must determine how much essential information is discarded (or never obtained) by the use of any given modification, and weigh this against any advantages thought to result because of the modification.

Random Notes on Miscellaneous Hematologic Techniques

The extreme value of a carefully performed, dependable "routine" hematologic examination cannot be overemphasized, for the majority of physicians place a great deal of confidence in its results, and depend on it as a guide for deciding what further studies may be needed. There is, however, developing an increasing awareness that the "routine" hematologic examinations are being carelessly and inaccurately performed in many laboratories and physicians' offices. The single example of the large number of patients with reported anemias, unresponsive to the whole armamentarium of antianemic therapeutic measures, who are being referred to hematology specialists and who, on further —possibly more exacting—study are found not to have been anemic at all is an alarming indication that many medical technologists are doing an inadequate job of the very familiar procedures of erythrocyte counting and hemoglobin-concentration determination.

Obtaining the Blood Sample: For most hematologic examinations capillary blood is unsatisfactory, although on rare occasions it may be the only readily available source of a blood specimen. Its use should always be reported to the physician so that he can take into account the inherent inaccuracies added to the results through its use. Dilution of the blood sample by added tissue fluids, or concentration by evaporation from exposure to air are the major sources of error, although the necessity to work much faster probably also is a large source of error. Venous blood should be obtained for all hematologic examinations whenever

possible. Its use permits checking results whenever indicated without additional inconvenience to the patient. When properly handled and thoroughly mixed, venous blood insures as truly a representative sample of the circulating blood as it is possible to obtain.

Blood to which an anticoagulant has been added is essential for many examinations. Dry potassium oxalate, in the exact ratio of 2 mg. per ml. of blood, has proved quite satisfactory for nearly all examinations, and has definite advantages over heparin or oxalate mixtures. It is not satisfactory, however, if used in greater concentrations. Potassium-oxalated blood is quite suitable for making morphologically excellent blood smears, if it is insured that these smears are made within one hour after the blood specimen is obtained, and no excess of the oxalate is present. It would seem, therefore, an unnecessary and inconvenient procedure to make "direct" smears from the syringe before the specimen is oxalated. Bone marrow specimens are more satisfactorily examined if they are oxalated, but it must be remembered that speed in mixing the oxalate and marrow is essential, since marrow tends to clot rapidly.

Hemoglobin Concentration Determination: Since they are available, an accurate, simple method for determining hemoglobin concentration should be employed. The various "bedside" techniques, such as the Tallqvist method, the copper sulfate gravity method, and use of one of the many different hemoglobinometers utilizing colored glass standards are not sufficiently accurate or reproducible to be of much value, and are likely to furnish misleading information, especially when attempting to classify an anemia. The results obtained with any of these may have an error as high as plus or minus 30 per cent.

Methods that can be recommended because of their accuracy and simplicity are those acid hematin, colorimetric techniques utilizing a liquid standard whose color value in terms of hemoglobin has been determined with a high degree of precision, usually by research techniques. The primary consideration in the determination of hemoglobin as acid hematin is the use of accurate equipment. Whenever possible a macrotechnique should be employed. Pipets that have been carefully recalibrated *to contain* a given quantity of blood are more satisfactory than pipets calibrated to deliver the desired quantity. If a photoelectric technique is utilized an accurate instrument is essential, since an inferior instrument can introduce considerable errors. Such instruments must be standardized for the method employed by the individual laboratory, and this standardization must be frequently checked, preferably daily, but certainly whenever the instrument is moved, or a filter or light is changed. The "standardizations" furnished by the manufacturers are in general inadequate.

Leukocyte counts of 100,000 or more per cu. mm. will produce a turbid solution of acid hematin, which results in an overestimation of the hemoglobin concentration, probably of the order of 5 to 10 per cent. So far a satisfactory way of correcting for this has not been found, but it is important to recognize that an overestimation may have occurred.

Erythrocyte Counting: There is at the present time no clinically practical, satisfactory substitute for the technique of counting erythrocytes using the hemocytometer. Although it is apparently used in many laboratories, the photoelectric, turbidimetric estimation of erythrocytes is not accurate in those cases in which the greatest accuracy is needed, the macrocytic anemias and the hypochromic microcytic anemias. Because the turbidimetric method measures particle size and not particle number, it overestimates the actual number of macrocytic erythrocytes and underestimates the number of microcytic erythrocytes. Thus, it can give color indexes in either type of anemia that will approach the normal color index, and therapy might then be instituted for a normocytic anemia, and the correct diagnosis be entirely missed, at least for a time.

The inherent errors of the hemocytometer count of erythrocytes are—or should be—well known to all medical technologists, and knowledge of their existence should foster the most scrupulous of technical manipulations, rather than carelessness and the indulgence in shortcuts. In a splendid article on this subject, Biggs and MacMillan[4] conclude: "the training of workers who make routine red cell counts should be a training in accurate counting rather than a training in achieving agreement between replicate counts." This places emphasis where it belongs and probably results in better agreement between replicate counts that is totally unbiased. Clean, dry, accurate equipment must always be used, and great precision must be achieved in making the dilution from a *well mixed* blood specimen that has not been left in an unstoppered container for any appreciable length of time. An uncontaminated, fresh diluting fluid that is not toxic to erythrocytes should be utilized. Of greatest importance, a sufficient number of cells (at least 300) should be counted, even though this necessitates counting more than one mount. In general, the error of the determination is proportional to the square root of the number of cells counted. In other words, in order to halve the inherent error of the method—the error that is due to chance distribution of the cells—it is necessary to count four times the number of cells. For an excellent discussion of the inherent errors of cell counting and the precautions that will help minimize these, one should consult the laboratory syllabus of Thomas Hale Ham.[5]

Leukocyte Counting: Here again use of the hemocytometer is the

most satisfactory method for estimating the number of leukocytes. The same inherent errors occur as do in erythrocyte counting, and the same careful attention to technique is necessary to minimize errors.[6] In leukopenic states it should be standard practice to make a 1:10 dilution of the blood (rather than the usual 1:20 dilution), and to count at least all nine squares of the chamber instead of the usual four squares.

The number of nucleated cells (including leukocytes and nucleated erythrocytes) per cu. mm. of specimens of oxalated bone marrow should always be determined by the usual technique of leukocyte counting. To estimate this as the "myeloid-erythroid" ratio from a centrifuged specimen of marrow in a graduated tube is a rough evaluation that is no simpler to perform than the somewhat more exact count of nucleated cells. Obviously, blood or marrow specimens that contain small clots are unsuitable for the determination of any cellular constituents.

If a patient is being followed closely with daily or very frequent counts—whether for a leukopenia or an anemia—in order to evaluate the effects of therapy, the use of the same pipets and other equipment for the daily counts on that patient will help minimize inaccuracies and will enable the physician to observe a "trend" with more confidence in its reality than he could otherwise justifiably take.

Differential Counting of Leukocytes: Only experienced technologists who have received specialized training in cell identification should be assigned the task of leukocyte differentiation. Even the most experienced morphologists frequently encounter cells they cannot readily identify; therefore, a technologist should have no hesitancy in reporting the presence of unidentified cells. A thin smear and an excellent stain are the primary prerequisites for accurate cell identification.

Volume of Packed Erythrocytes (The Hematocrit): This simple procedure is invaluable in the differentiation of anemias, and in the determination of the total blood volume. The hematocrit tube should be centrifuged in an instrument capable of attaining a force of over 2,000 times gravity (about 3,000 r.p.m.). The most important consideration is that the hematocrit tube be spun long enough and with sufficient force so that a *constant* packed cell volume is obtained. This cannot be achieved if all specimens are centrifuged for a set interval of time, since erythrocyte sedimentation rates differ, which directly influences the centrifugation time required to reach constant packed cell volume. Chaplin and Mollison[7] have published an interesting discussion of the percentage of plasma that is trapped in the erythrocyte column in the hematocrit, and have presented a calibration curve for correcting the observed packed cell volume for trapped plasma.

Reticulocyte Counting: When anemia is present the relative rate

of erythrocyte production should be determined at frequent intervals in order to evaluate the effects of therapy. The reticulocyte count furnishes the most reliable criterion of an increased or decreased rate of erythrocytopoiesis, and therefore is a valuable aid in diagnosis and prognosis.

A reliable technique, yielding the highest number of reticulocytes when checked against other methods on the same blood specimen, is the Osgood-Wilhelm[8] method, using a freshly filtered 1.0 per cent solution of brilliant cresyl blue in 0.85 per cent sodium chloride solution to stain the reticulum. The stain is mixed in equal parts with the blood specimen in a test tube and allowed to stand for about 15 minutes. Thin smears on glass slides are then made from the blood-stain mixture, and it is not necessary to counterstain in order to count the reticulocytes microscopically, although a reduced light is helpful. If the reticulocyte count is less than 5 per cent, at least 1,000 erythrocytes should be examined.

A recent modification[9] of the standard counting technique, using a Miller disk in the eyepiece of the microscope, appears to have certain advantages. The Miller disk superimposes a square rectangle on the microscopic field that contains a smaller square within it, the two squares having an area ratio of 1:9. All erythrocytes are counted in the small square and all reticulocytes in the large square. A minimum of twenty large, adjacent squares are counted and the reticulocytes are reported as a percentage of the erythrocytes, computed as follows:

$$\frac{100 \times \text{reticulocytes in large squares}}{\text{erythrocytes in small squares} \times 9}$$

Autoagglutination of Erythrocytes: A frequent, serious complication of chronic lymphocytic leukemia and of some of the lymphomas is acquired hemolytic anemia. Specific therapy for the leukemia of lymphoma has no effect on this type of anemia, so it is important that its presence be recognized early so that appropriate therapy can be instituted. A simple laboratory observation can be helpful and is quite reliable in determining quickly whether autoagglutination of erythrocytes is present, which is a characteristic feature of many cases of acquired hemolytic anemia. The tube containing a sample of the patient's blood is inverted once and then righted; as the blood flows down the sides of the tube it is examined using a 10 X reversed eyepiece from the microscope, or a hand lens. This is sufficient magnification to observe the small aggregates of agglutinated erythrocytes, and the degree of autoagglutination can be graded on a one to four plus scale. Heavy rouleau formation cannot be distinguished from true autoagglutination when examined in this way, but a moist cover-slip preparation of a tiny drop of blood examined microscopically will reveal whether the aggregates of cells have the

characteristic "stacked-coins" appearance of rouleaux, or the clumps of autoagglutination.

Lee and White Coagulation Time: It is essential that the determination of blood coagulation time be done on venous blood, which has been obtained with a minimum of trauma and stasis by a skillful venipuncture. Capillary blood is totally valueless for determining an accurate coagulation time. The syringe, needle, and the test tubes used should be rinsed with sterile physiologic saline just before making the venipuncture. The test tubes used should be chemically clean and should have been measured ahead of time to determine that they have an internal diameter of exactly 8 mm. A tube of any other diameter is acceptable so long as the range of normal for that size has been determined, and only the one size is used consistently. It facilitates manipulations if the tubes have been previously calibrated to contain 1 ml., a line indicating this volume being etched on the tube. Some modified techniques specify the use of 2 ml. of blood, but again the range of normal using this volume must be determined, since the relationship of surface area to the volume of blood is a factor influencing the coagulation time.

The first few milliliters of blood that enter the syringe are unavoidably contaminated with some tissue thromboplastin, introduced when the needle went through the skin and underlying tissue into the vein. Therefore, when the blood is removed from the syringe the last few remaining milliliters should not be used for any coagulation time study, but should be either discarded or used for other purposes.

Set up three 8 x 75 mm. tubes. As soon as a clean venipuncture has been accomplished and the instant blood is seen to appear in the tip of the syringe, a stopwatch should be started by an assistant, or the time very accurately noted. As soon as sufficient blood has been obtained, come out of the vein cleanly and immediately remove the needle from the syringe. Introduce *exactly* 1 ml. of blood into each of the three 8 x 75 mm. tubes, allowing the blood to flow gently and slowly down the sides of the tubes, so that bubbles are not formed. Air bubbles in the tubes or in the syringe tend to hasten the coagulation process. Every 30 seconds the first tube is gently and only slightly tipped (at an angle of not more than 30°) to determine if the blood will still flow. When the first tube can be inverted without the blood flowing, record the time, then tip the second tube in a similar manner. The coagulation time is arbitrarily recorded as the time elapsed from the initial appearance of blood in the syringe until the second tube can be inverted. Using this technique, without modifications, the normal (95 per cent) range is between 5 and 15 minutes in our laboratory. The test is performed at room temperature, and this may be a factor in giving a rather wide range of normal.

Some investigators feel that the range can be narrowed if the test is performed at a constant temperature, preferably in a 37.5° C water bath. However, in clinical practice this is not always convenient and the refinement may have only research significance.

Clot Retraction Time: This is primarily a test of thrombocyte function and is generally determined concurrently with the coagulation time test. It is a valuable guide in following the response to therapy in any patient with thrombocytopenia, since it is far more reliable than thrombocyte counts by any present clinical method. The third 8 x 75 mm. tube of the Lee and White coagulation time test is used to evaluate the speed and degree of clot retraction. The tube is not tipped or agitated, but is corked and placed upright in the incubator as soon as coagulation occurs. It is examined after being in the incubator one hour for beginning clot retraction. If the history or other findings suggest thrombocytosis, examine the tube after one-half hour. If clot retraction is started in one hour (as it should be normally), examine the tube again after 18 hours, to determine that clot retraction is complete within that time. If clot retraction has not started after one hour, examine the tube hourly thereafter as long as is conveniently possible, noting the time of beginning retraction if it occurs.

A modification of the clot retraction test that furnishes semiquantitative as well as additional data has been proposed by Aggeler, Lucia, and Hamlin.[10] In addition to the 8 x 75 mm. tube, set up a graduated centrifuge tube—such as the 4 ml. hematocrit tube—that has also been rinsed in saline and to which has been fitted a cork holding a wire loop that can be inserted well into the blood in this tube. Introduce 3 to 4 ml. of blood into the calibrated centrifuge tube and record the exact amount before inserting the wire loop. This tube is placed in the incubator and examined for clot retraction in the same manner as the smaller tube. In some normal clot retraction tests there may be a small layer of untrapped erythrocytes in the bottom of the tube. This should always be looked for and the volume of untrapped erythrocytes estimated, for a significant increase in the number of untrapped erythrocytes is the only abnormal laboratory finding in the rare hemorrhagic pseudohemophilic type of thrombasthenic purpura.

By lifting the clot out of the graduated centrifuge tube with the wire hook, one can quantitate the number of loose erythrocytes remaining, and can also roughly estimate the firmness or fragility of the formed clot. Dissolution of the clot after it has formed and retracted should also be looked for and reported, since rare cases of hemorrhagic disease due to fibrinolysins occur.

Thrombocyte Counting: No presently available method for enumerating thrombocytes, having any reasonable clinical degree of

reliability or reproducibility, can be recommended. The clot retraction time test and a careful examination of a thin, Wright's stained blood smear furnish reliable, qualitative information about thrombocyte function, approximate number, and morphologic characteristics. A recent report,[11] utilizing the phase microscope and venous blood in siliconed equipment, may prove to be worth while, but this technique needs further evaluation at the clinical level.

Eosinophil Counting: As has been pointed out by Speirs[12] in a recent review article, the direct procedure for counting eosinophils using any of the special diluting fluids have inherent difficulties that should be known to everyone using them, and this paper should be read by anyone wishing to learn the "causes and cures" of the inherent problems.

The Peroxidase Stain: This valuable stain should be used more frequently in clinical laboratories, particularly whenever there is any difficulty in identifying the series to which leukocytes belong. It is especially valuable in distinguishing progranulocytes from prolymphocytes, for the former are always peroxidase positive and the latter always negative. It frequently is helpful in differentiating atypical promonocytes from prolymphocytes or progranulocytes, but here it is not invariably reliable, for promonocytes may or may not be peroxidase positive. Experience with this stain is necessary to feel secure in differentiating immature cells of the granulocytic series from immature cells of the monocytic series, when the latter are peroxidase positive.

A satisfactory technique for making the peroxidase stain is a modification of the Washburn method. Thin, air-dried smears of blood or marrow should be made within one hour after drawing the specimen, and should be stained within 12 hours, unless carefully protected from light and air. Flood the slide with solution I and allow to stand 1 to 3 minutes, then add directly about 6 to 8 drops (0.4-0.5 ml.) of solution II, mix thoroughly and allow it to stand 3 to 4 minutes. Wash the stain off with tap-water, floating the stain off first and then washing thoroughly for 1 to 1.5 minutes, and allow the smear to air-dry. Counterstain with Wright's stain in the usual manner, but allow it to stain for 40 to 60 minutes after adding buffer phosphate of pH 6.4.

Solution I: 0.3 Gm. benzidine base (benzidine dihydrochloride is not satisfactory in our experience) is dissolved in 99.0 ml. of 95 per cent ethanol and 1.0 ml. of saturated (36 per cent) sodium nitroprusside solution is added. This stain keeps for 8 to 10 months. In practice it has been found best to add the stain to the slide through a filter.

Solution II: Eight to ten drops (approximately 0.5 ml.) of 3.0 per cent hydrogen peroxide are added to 25 ml. of distilled water. This must be made up immediately before use. Poor results usually indicate that the stock bottle of hydrogen peroxide is too

old; it should be refrigerated at all times.

"Buffy Coat" Smears of Blood or Marrow: Stained smears of concentrated leukocytes are frequently helpful in finding scarce, pathologic cells more readily. The "buffy coat" is obtained by slow centrifugation of the specimen—not more than 500 to 800 r.p.m.—for about 5 minutes, just enough so that the layer of leukocytes is loosely deposited on top of the erythrocytes. The leukocytes can then be selectively aspirated from the specimen using a capillary pipet, placing small drops on glass slides and smearing in the usual manner. These smears are then stained with Wright's stain, but a longer staining time is usually required, frequently as much as 20 to 30 minutes. This technique is very satisfactory for making "L. E." cell preparations, no matter whether oxalated, clotted or defibrinated blood is used.

Summary

Some fundamental considerations important to the evaluation of and attainment of accuracy in clinical laboratory procedures in hematology are presented.

In a random manner, a number of basic hematologic techniques are presented and discussed, but no attempt at completeness in covering this field is made.

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RECENT DEVELOPMENTS IN CLINICAL CHEMISTRY

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Anthrone Reagent for Carbohydrate Determinations

In 1946, Dreywood[1] described a qualitative reaction in which a 0.2% solution of anthrone* in 95% sulfuric acid gives a green color with carbohydrate material. The reaction is very sensitive, i.e. forty times more sensitive than iodine for the detection of starch. One part of starch in 900,000 parts of water will still give the anthrone reaction.

The reaction was studied by Satler and Zerban[2,3] who found the anthrone in sulfuric acid reacted with sugars, polysaccharides, glycosides, carbohydrate ethers and esters. The anthrone reaction requires the initial formation of furfural or a derivative of furfural by splitting out water from the carbohydrate by the action of the concentrated sulfuric acid. A red color is obtained when anthrone reagent reacts with ascorbic acid, proteins, and uric acid in contrast to the green color obtained with carbohydrates.

Morris[5] was the first to apply the anthrone reaction to the quantitative determination of carbohydrates such as blood glucose, lactose in milk, glycogen in blood and liver, and urine sugar. Because no additional heat is used for the reaction other than that evolved in the mixing of the concentrated acid of the reagent and the water of the carbohydrate-containing solution, Morris points out that the size and shape of the reaction tube is important. Optimum conditions call for four or five ml. of carbohydrate solution and twice this volume of anthrone reagent in a tube 19 to 25 mm. in diameter. (Evelyn colorimeter tubes are 19.5 mm. in diameter.) Less color is obtained in narrower or larger tubes, so if the Klett-Sumerson instrument is to be used, the reaction should be carried out in 19-25 mm. diameter tubes and poured into the Klett tubes after the color development period of fifteen minutes. The volume can be varied to suit the colorimeter used, provided the volume of the anthrone reagent is no less than twice the volume of the carbohydrate solution. Because the reagent darkens with time of storage, a blank and standard must be included with each series of unknowns. With the Evelyn instrument Morris used the 620 filter for a range of 8 to 200 micrograms and the less sensitive 540 filter to extend the range to 500 micrograms of glucose per tube. The developed color is stable for days if stoppered with non-rubber stoppers and refrigerated.

Morris' method applied to the determination of blood sugar was studied extensively by Durham et. al.[5] of the U. S. Public Health Service. They obtained the same blood glucose value with trichloracetic acid, Folin-Wu, or Somogyi barium

* Eastman Organic Chemicals.

hydroxide-zinc sulfate protein-free filtrates. They used the 620 filter exclusively with the Evelyn. Our own experience, using the 540 filter with the Evelyn, is that the barium hydroxide-zinc sulfate protein precipitant gave the best agreement with the Benedict blood glucose method, while the Folin-Wu filtrate gave higher results with the anthrone reagent. This may be due to the fact that uric acid, which gives a red color with the anthrone reagent, is measured sufficiently with the 540 filter to warrant its removal with the proteins. The use of the 620 filter minimizes the effect of the interfering color. Durham et al. found recovery of added glucose ranged from 98 to 101%. Comparison with an accepted standard method, the Nelson-Somogyi, on the 125 bloods gave the results of 116.6 ± 3.82 mg. % with the Nelson-Somogyi method and 118.4 ± 3.59 mg. % with the anthrone reagent.

They report the following advantages to the use of the anthrone reagent for blood glucose:

1. A single reagent is added to the protein-free filtrate.
2. No heating is required.
3. Sufficient blood can be obtained by finger prick.
4. Results with the anthrone reagent are in close agreement with an accepted standard method and recovery of added glucose is good.

The anthrone reagent is also used by Kibrick et. al.[6] instead of a copper reducing glucose method for the determination of amylase activity in blood plasma and urine.

Although the blank and standards will take care of the darkening of the reagent with time, some may find this discoloration of the reagent a disadvantage. Loewus[7] reports that the darkening of the reagent is inversely related to the purity of the sulfuric acid used. He has worked out a two step reagent, in which the anthrone dissolved in ethyl acetate and the sulfuric acid are added separately to the carbohydrate-containing solution.

Direct Titration of Calcium and Magnesium in Blood Serum

The Clark Collip modification of Kramer Tisdall permanganate titration for serum calcium has been standard practice for blood serum calcium. However, as evidenced by the survey made in Pennsylvania by Belk and Sunderman,[1] the method often is reported from the laboratory in error. These authors reported a range of 7 mg. % to 26.6 mg. % on a calcium specimen of 12.6 mg. % sent to the surveyed laboratories with 30 out of 44 laboratories reporting results outside the accepted variation range of ± 0.5 mg. %. There is no reason to believe that this situation does not prevail the country over wherever proper pains are not taken to control the method. Our own experience with students has been that the permanganate calcium method re-

quires a longer "learning period" than any other clinical chemical procedure. When proper precautions are taken in the washing of the precipitate and in the standardization of the reagents, the method is reliable. The other disadvantages of the method are the requirement for at least 4 hours to precipitate the calcium oxalate, which inconveniences the treatment of hypoparathyroid tetany, and the fact that it requires more serum than can be obtained with ease from pediatric patients.

In an effort to find a method which would obviate these difficulties, many authors are now proposing methods for calcium and for calcium plus magnesium in blood serum using the chelation principle. These methods are based on the work of Schwarzenbach, et. al.[2,3] on the formation of complexes of the alkaline earth metals with di-sodium ethylene diamine tetra-acetate (EDTA).* When the metal ion is bound by the chelating agent EDTA, it can no longer give an effect on an indicator which it could give when it was in the ionic state.

Murexide (ammonium purpurate) is the specific indicator for calcium. Ionic calcium gives a pink color to the indicator which reverts to an orchid-purple when the calcium is all bound by the EDTA.

The indicator, Eriochrome Black T, is used for the calcium plus magnesium titration. The behavior of Eriochrome Black T varies with pH.[4] At pH values below 6.3 the indicator is red in color; at all values between 6.3 and 11.5 the color of the indicator is blue. At pH 10, however, calcium and/or magnesium ions in the solution cause the indicator to assume the red color normally exhibited at pH 6.3 or lower. At the endpoint of the titration with EDTA, the blue color of the indicator is restored. The chelation occurs quantitatively, so the volume EDTA used in the titration is directly related to the amount of calcium and magnesium present. Sodium and potassium are not chelated by the EDTA.

The method has become standard practice for the determination of calcium hardness (murexide) and total hardness of water,[5] and recently several authors have applied the method to blood serum.[6,7,8,9,10,11] Friedman[8] reports the method is capable of ten times the precision of previously used methods. Other authors report good agreement with the permanganate method. The authors differ in details of their procedure such as pH and buffers.

All authors are equally enthusiastic about the titration of calcium plus magnesium with EDTA using Eriochrome Black T. The color change from pink to violet blue on the calcium titration using murexide as the indicator evidently is not nearly as easy to see. For this reason Sobel and Hanok[9] prefer to use the

* Also known by various trade names, i.e., Versene, Complexone.

indicator Eriochrome Black T only and to determine magnesium separately by the colorimetric titan yellow procedure[12,13] and calculate calcium by difference. Because magnesium values vary from the normal very rarely, these authors also suggest subtraction of a normal value for magnesium from the sum of calcium plus magnesium. The endpoint can also be observed with a photoelectric colorimeter selecting a filter which gives the greatest change in extinction between one color of the indicator and the other.[10]

The EDTA method gives an obvious saving in time because no precipitation of calcium oxalate is required. It also offers the improvement in precision mentioned earlier. However, the EDTA titration is not free of interference by other material which might be present. The color of bilirubin in a jaundiced serum can alter the shade of the indicator at the endpoint. This difficulty could be eliminated by using a photoelectric colorimeter to determine the endpoint. Hemolyzed serum presents even more of a problem. Iron is also chelated by EDTA, and the presence of magnesium from red blood cells would increase the serum magnesium value. There is no calcium in red cells, and the Buckley et. al.[7] were able to avoid interference from iron in the calcium titration by including potassium cyanide in the solution to tie up the iron and prevent its chelation by EDTA. They report that the potassium cyanide also eliminates interferences by copper and zinc. Mason[14] mentions interference by phosphate in the determination of calcium in plant material. The error here is due to the precipitation of calcium phosphate at the very alkaline pH of the titration. None of the authors who have worked with serum report difficulty from this source. The explanation may lie in the fact that the plant material was converted to inorganic ash before titration, while the serum is still stabilized by the proteins. Mason solved the problem through the use of ion exchange resins.

The EDTA method is limited only by the size burette used in the titration in so far as volume of serum required is concerned. The volumes range from the method of Sobel and Hanok[9] using 0.02-0.05 cc. serum and a microliter burette to that of Elliot[11] who expanded the method back to macro proportions requiring 2 cc. of serum and ordinary laboratory burettes with less than five minutes required per determination.

The method is obviously still in the developmental stage, though it shows considerable promise. The final kinks can be worked out only after the method has been put to the experience of many different laboratories. Perhaps, those of our members who have been fortunate enough to have used the method would like to share their experience with the group either by writing their comments as a letter to the editor or as a journal article.

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SOME RECENT DEVELOPMENTS IN LIVER FUNCTION TESTS*

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Clinicians, pathologists and chemists working together have produced a whole battery of new liver function tests during the past ten years. They have described these tests in medical or scientific journals that the clinical laboratory technician seldom sees. It is the purpose of this article to discuss some of these new liver function tests from the point of view of the laboratory technician.

The new tests are run on blood serum and depend on the colloidal nature of the serum proteins. Scientists have already identified six or eight different serum proteins and it is likely that others will be discovered. These proteins are made up of huge molecules, a thousand or more times as large as a sodium chloride molecule. All of the molecules are made up of amino acids, so that they are much alike in composition. They are put together differently, however, and differ in size and shape. They are loosely bound together in the serum to form aggregates or groups that are not large enough to be seen under the microscope but are too large to form a simple solution. They are carried in solution because they all carry the same kind of electric charge, so that they repel each other. This kind of solution is called a colloidal solution.

The serum proteins can act as either acids or bases, or they can be electrically neutral like sugar. The way they act depends on the pH of the medium in which they are dissolved. At a certain pH range, called the isoelectric range, they are electrically neutral. If the medium is more acid than the isoelectric range, the proteins become cations; that is, they carry a positive charge. If the medium is more alkaline than the isoelectric range, the proteins become anions and carry a negative charge. The isoelectric range of the serum proteins is around pH 5.

The new liver function tests take advantage of these facts. The flocculation tests—cephalin-cholesterol flocculation and thymol turbidity and flocculation tests—depend on the fact that the serum proteins form complexes with other colloidal particles at certain pH ranges. The zinc sulphate turbidity test and others of that sort depend on the fact that the serum proteins will combine with heavy metals—zinc, iron, copper, mercury, etc.—in alkaline solution to form precipitates. The gamma globulin precipitation test and similar tests depend on the fact that the serum proteins can be precipitated more or less as individual

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proteins by graduated additions of neutral salts at a pH near their isoelectric range.

Although the facts on which the new tests are based have been known for a long time, the development of the tests depends on, and had to wait for, three other technical advances. These are the Warburg respirometer, the Tiselius electrophoresis apparatus, and the needle biopsy technique.

Medical men have suspected for a long time that the liver provides the raw materials for the body's metabolism. Today, thanks to the Warburg apparatus, they know that this is true. Scientists, using the Warburg apparatus, have discovered more than four hundred enzyme systems in the liver cells. These enzymes produce the basic raw materials for every phase of metabolism. The scientists have identified some of these materials as the plasma proteins, and have explained how the liver produces them. They have also shown that some of these protein fractions, which are carried in the blood plasma, may be altered when the liver is not working properly. This fact is a basis of the new liver function tests. The doctor can study a patient's liver function by having tests run on the blood serum. These tests determine the various serum proteins directly, or their balance indirectly.

Even with the knowledge gained by means of the Warburg apparatus we could not have developed the new liver function tests if we had to depend solely on the chemical separation of the serum proteins. Our only means of separating them up to fifteen years ago was by chemical separation very roughly into groups. We could tell by means of immunological and other tests that our groups contained mixtures of proteins. We could identify some of the different proteins in our mixture, but we could not find out how much of each protein was present. The Tiselius electrophoresis apparatus separates out the proteins from a mixture of them and tells the relative amount of each.

Tiselius announced the invention of his apparatus in 1937. He claimed that he had separated the albumin from the globulin fraction. He had also separated the globulin fraction into three sub-groups which he called alpha, beta and gamma globulins. The apparatus is based on the fact that proteins carry electric charges in solutions either more acid or more alkaline than their isoelectric range. If two electrodes bearing opposite charges are immersed some distance apart in a solution either more acid or more alkaline than the isoelectric range, the protein molecules move toward the electrode bearing a charge opposite to their own. The lighter the molecule the faster it moves, so that the protein fractions become separated after a time. The separated proteins are then measured in the apparatus or they are removed separately from it. The Tiselius apparatus is an expensive re-

search instrument not suited to routine clinical use. It has been used to check and improve chemical separation of the serum proteins and to explain the mechanism of some of the new liver function tests.

The third technical advance is the needle biopsy technique. This technique makes it possible for the doctor to remove a small piece of liver tissue without opening the patient's abdomen. The pathologist can then make a microscopic study of the biopsy specimen and get an idea as to what is wrong with the liver. This direct examination of the liver tissue is the most reliable liver function test. It does not agree with the clinical findings one hundred per cent, but it agrees with them more frequently than does any of the other liver function tests. It has been helpful in developing the other tests by checking their findings against the liver cells.

Most of the new tests aim to detect increases in gamma globulin, since an increase in this protein is considered by many to be one of the first signs of liver disease. Other tests detect changes in serum albumin or in lipoprotein. Since most of these tests overlap in purpose, we use only two of them—the cephalin-cholesterol flocculation and the thymol turbidity tests—routinely at the Rees-Stealy Clinic. We also use the gamma globulin turbidity test of Popper as a quantitative measure of gamma globulin, and the zinc sulphate test of Kunkel when the serum is icteric.

A brief review of the literature is the best way to summarize the development and significance of these tests. We have selected only a few of the many papers that have been written on the subject and have chosen only one or two significant points from each.

Gray[1] started the ball rolling in 1942. He was the first to test fractions of serum proteins, separated by electrophoresis, with a colloidal reagent. He used the colloidal gold test and reported that gamma globulin is the flocculating agent. Albumin was an inhibiting agent.

The next year, 1943, Kabat, Hanger et al.[2] confirmed Gray's results and extended them to Hanger's cephalin-cholesterol test.

Moore, Hanger et al.[3] established a very important point in 1944. These authors observed that the albumin from the serum of patients with "diffuse parenchymatous liver disease" (such as infectious hepatitis) does not inhibit the flocculation by gamma globulin as much as the albumin from the serum of normal persons. There was no difference in the flocculating power of the gamma globulins from the two kinds of serum. Albumin, from whatever source, lacks flocculating power in the cephalin-cholesterol flocculation test, and gamma globulin, from whatever source, always has this power. Albumin shows qualitative differ-

ences in inhibitory power, but gamma globulin shows no qualitative differences in flocculating power.

Along the same line, Moore, Hanger et al.[4] explained the mechanism of the positive cephalin-cholesterol floculation test as follows. The flocculating effect of gamma globulin occurs (a) when the gamma globulin is increased so much that a normal amount of albumin cannot inhibit it; (b) when the *quantity* of albumin is decreased so much below the normal level that it cannot inhibit normal quantities of gamma globulin; (c) when the *quality* of the albumin is deficient, so that its inhibitory power is partly lost.

While Moore, Hanger et al. were studying the cephalin-cholesterol flocculation test in this country, MacLagan[5] proposed the thymol turbidity test in England in 1944. He used proteins as standards as in the Addis counts.

The next year, 1945, Recant, Chargaff and Hanger[6] compared the cephalin-cholesterol flocculation test with MacLagan's thymol turbidity test. If they extracted the lipides from hepatitis serum a formerly positive thymol turbidity test became negative, but the cephalin-cholesterol flocculation test remained just as positive as before. Gamma globulin isolated from either normal or pathological serum does not give a positive thymol turbidity test, but does give a positive cephalin-cholesterol flocculation test. Albumin from normal serum in sufficient amounts inhibits the thymol turbidity tests, but albumin from hepatitis serum does not.

Chen and Thompson[7] reported on the mechanism of the thymol turbidity test in 1947. They used the Tiselius apparatus to study the proteins left in the supernatant serum after precipitation of the serum proteins by the thymol reagent. They observed a questionable decrease in gamma globulin and a marked decrease in beta globulin.

Earlier, in 1946, Hanger[8] had stated that the determining factor in a positive cephalin-cholesterol flocculation test lies in alteration of the albumin fraction, but that the positive thymol turbidity test depends on changes provoked by gamma globulin in the presence of lipides. He had observed that gamma globulin isolated from hepatitis serum gives a negative thymol turbidity test, but that the test becomes positive if lipides or normal whole serum is added.

Kunkel and Hoagland[9] stressed the importance of lipides to a positive thymol turbidity test, but considered gamma globulin the essential factor. They thought that lipides influence the intensity of the test.

Bauer[10] studied the flocculation tests with protein fractions isolated with the Tiselius apparatus. No individual fraction gave a positive thymol turbidity test. Lipides added to either beta or

gamma globulins gave a positive thymol turbidity test.

The most important points brought out in the ten papers are the following:

(a) Albumin has no flocculating power. Its only effect in the flocculation tests is an inhibitory effect.

(b) The inhibitory effect of albumin on the flocculation tests is conditioned by quality as well as quantity. Albumin from normal serum has greater inhibitory power than that from hepatitis serum.

(c) Gamma globulin is a definite flocculating agent.

(d) Gamma globulin, from whatever source, seems to have the same flocculating power. Its power depends entirely on its amount and on the inhibitory power of the albumin.

(e) Lipoproteins in which lipides are combined with beta and/or gamma globulins are necessary for a positive thymol reaction. The phenolic group of the thymol seems to combine with the lipides and proteins to form phenol-lipide-protein complexes.

The latest editions of some of the clinical laboratory manuals contain descriptions of the cephalin-cholesterol flocculation and thymol turbidity tests. We perform these tests much as they are described in Hepler's manual.* We recommend this manual not to disparage others but because, in addition to having an excellent coverage of general clinical laboratory procedures, it devotes a whole chapter to liver function tests.

Hepler describes the tests as they are given in the original papers or in early modifications of original tests. We shall describe modifications not mentioned by Hepler that have been suggested to us by later papers or our own experience.

We have incorporated a few modifications in the cephalin-cholesterol flocculation test described by Hepler. We use one-half the quantities used in preparing the original cephalin-cholesterol emulsion and a different method of preparation. We prepare the emulsion as follows:

Transfer 15 mls. of distilled water to a 50 ml. conical flask and mark the level of the meniscus. (We have found it convenient to scratch marks at the 15 ml. level in the glass of 2 or 3 flasks that we reserve for preparing and storing cephalin-cholesterol emulsions.) Add 5 more mls. of water to the 15 mls. in the flasks. Add 5 mls. of ether to the contents of a freshly opened cephalin-cholesterol antigen bottle or replace any ether that may have evaporated from a previously opened bottle. Stopper the bottle and shake the contents until all of the antigen dissolves. Transfer 0.5 ml. of the antigen solution to the 20 mls. of water in the conical flask at room temperature and mix by swirling the flask. Mark the level of the antigen solution on the antigen bottle so that any evaporated ether may be replaced before removing the next sample. Place the flask containing the emulsion in an oven heated to about 110° C. and drive off the ether by evaporating, with occasional shaking down to the mark on the flask. Stopper the flask and set it aside to cool.

We set up our cephalin-cholesterol flocculation test as follows:

*O. E. Hepler: *Manual of Clinical Laboratory Methods*. 4th Ed., 3rd Revised Printing. Springfield, Ill. Charles C. Thomas, 1951.

Transfer 2.1 mls. of 0.85% sodium chloride to one Kahn tube and 2.0 mls. to as many other Kahn tubes as there are tests to be run. Mark the tube containing 2.1 mls. "C" for "control" and the other tubes with the patients' initials. Transfer 0.1 ml. of each serum specimen to the appropriately marked tube and mix serum and saline by tapping the tube. Add 0.5 ml. of cool emulsion to each tube, mix the contents, stopper the tubes with cotton and set them away in a dark cupboard at room temperature. Read after 24 and 48 hours. Grade the tests as follows:

(a) No visible difference against light from control—0.
(b) Fine, scattered precipitate visible against light—1 plus.
(c) Some floccules but no apparent change in emulsion—2 plus.
(d) Floccules and occasional clear patches in emulsion—3 plus.
(e) Floccules on bottom, clear solution above—4 plus.

Hepler says that the emulsion will keep three days in a refrigerator, but we have observed appreciable differences in flocculation when the same serum was tested with fresh and three-day-old emulsions made with antigen taken from the same bottle. We do not know whether the variation was due to the emulsion, the serum, or both, or whether the difference came about gradually or suddenly. Three-day-old emulsions may be safe enough for routine use, but we prefer to make up half quantities and use the emulsions the day they are made.

Hepler also says that the cephalin-cholesterol flocculation test must be set up within four hours after the blood is drawn, and we agree with this estimate. Serum specimens vary in the length of time they can stand before beginning to lose flocculating power, but we have no knowledge as to how soon such a loss may begin. All of the specimens we have tested lost flocculating power if held in the refrigerator overnight. Hence all cephalin-cholesterol flocculation tests should be run a few hours after the blood is drawn.

We perform the thymol turbidity test essentially as described by Hepler except that we use a modified thymol solution. De la Huerga and Popper[11] observed that the thymol content of solutions prepared according to MacLagan varies from batch to batch, and that this variation in thymol content causes variation in the sensitivity of the test. They devised a method for making solutions of constant thymol content. They prepare the solution as follows:

(a) Prepare one liter of barbiturate buffer having a pH of 7.55. (See Hepler's manual.)
(b) Dissolve 10 grams of pure thymol in about 75 mls. of 95% ethyl alcohol and dilute to 100 mls. with alcohol.
(c) Add 5.0 mls. of the 10% alcoholic thymol solution to about 800 mls. of buffer solution, mix and dilute to one liter with buffer solution. This is the stock solution.
(d) Add 0.5 ml. of 10% alcohol thymol solution to about 75 mls. of stock solution, mix thoroughly and dilute to 100 mls. with stock solution. This is the working solution.

The cephalin-cholesterol flocculation and thymol turbidity tests were thought at one time to measure gamma globulin. We

know now that albumin affects the cephalin-cholesterol flocculation fully as much if not more than does gamma globulin, and that lipides and beta globulin affect the thymol turbidity even more than does gamma globulin. Information obtained with the Tiselius apparatus indicates that an increase in gamma globulin is one of the earliest signs of liver disease. Medical men have searched, and are searching, for other methods of determining gamma globulin that can be used routinely in the clinical laboratory. The two methods described in the following paragraphs have achieved greater prominence than the others.

Kunkel[12] tried to combine gamma globulin with heavy metal ions in buffered solution to form a precipitate that could be measured turbidimetrically. He discovered that the precipitate formed with zinc sulphate correlates best with gamma globulin as determined with the Tiselius apparatus. We perform the zinc sulphate turbidity test as follows:

Reagent: Dissolve 24 mg. of $ZnSO_4:7H_2O$, 280 mg. of barbituric acid and 210 mg. of sodium barbiturate in distilled water, and dilute to one liter.

Procedure: Add 0.1 ml. of serum to 6.0 mls. of reagent, allow to stand 30 minutes to one hour, mix by inversion and read in a photoelectric colorimeter using an orange filter.

Calculation: Use the standards and scale developed by Shank and Hoagland for measuring thymol turbidity. (cf. Hepler)

Later, De la Huerga and Popper[13] adapted Wolfson's[14] gamma globulin reagent to a turbidimetric technique. We perform their gamma globulin turbidity test as follows:

Reagent: Dissolve 189 grams of ammonium sulphate and 29.3 grams of sodium chloride in distilled water and dilute to one liter.

Procedure: Add 0.1 ml. of serum to 5.0 mls. of reagent, allow to stand 30 minutes to one hour, mix by inversion and read in a photoelectric colorimeter using an orange filter.

Calculation: The gamma globulin turbidity, the thymol turbidity and the zinc sulphate turbidity tests are all measured in arbitrary units devised by Shank and Hoagland. They all use the scale described in detail by Hepler as part of the thymol turbidity test. De la Huerga and Popper change units to grams per cent of gamma globulin by means of the following formula: G. G. in gms./100 mls. $= \frac{\text{units}}{5.8}$ plus $0.02(20 - \text{units})$

The new turbidimetric gamma globulin tests have not been thoroughly studied yet, but some interesting information about them has been published. We shall summarize some of this information briefly.

(a) De la Huerga and Popper[15] compared the results obtained with the gamma globulin and zinc sulphate turbidity tests with gamma globulin determined with the Tiselius apparatus. They reported fair correlation between the two turbidity tests, fair correlation between the gamma globulin turbidity and the electrophoretic results, and not so good correlation between the zinc sulphate turbidity and electrophoresis.

(b) Ricketts et al.[16] reported that both gamma globulin and zinc sulphate tests correlate fairly well with electrophoresis in

normal and acute hepatitis sera, but that they both give low results for gamma globulin in both portal and biliary cirrhosis.

(c) Popper et al.[17] compared the zinc sulphate turbidity with the thymol turbidity test. They observed that the ratio of thymol turbidity units to zinc sulphate units is higher in acute hepatitis than in cirrhosis, and that the zinc sulphate units, usually elevated in liver disease, are not elevated in extra-hepatic jaundice.

The new tests need further study to correlate them with clinical findings in various stages of the liver diseases. They show promise of being useful both in differential diagnosis and in detecting early and/or minimal liver disease. We are using these new liver function tests in a study now in progress at the Rees-Stealy Clinic. We hope to be able to contribute to a further understanding and appreciation of them at a later date.

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SOME PRACTICAL INEXPENSIVE IMPROVEMENTS ON BLOOD CULTURE TECHNIQUES

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Expensive equipment is not necessary in order to obtain satisfactory results with blood cultures. The following suggestions are modifications suggested in the technique of Castenada[1] and can be made with materials easily available to all laboratories.

Methods

Eight ounce hexagonal narrow mouthed pyrex nursing bottles that can be sealed with gum rubber caps are used. These rubber caps are easy to handle as they have a projecting tab on the side and are heavy enough to withstand several sterilizations and needle punctures and still maintain adequate sealing. When the culture medium in the bottle is autoclaved the rubber caps are prevented from blowing off by a simple device that allows the expanding air to escape yet maintains the caps in position. This consists of two pieces of wood two inches wide and two feet long, held together with ordinary hooks (Fig. 1). When the bottles cool a partial vacuum is formed. An alternate procedure is to puncture the cap before sterilization with a needle airway (Fig. 2) that consists of a syringe needle which has attached to the hub a short length of rubber tubing which is packed with cotton. This allows free interchange of gases yet maintains the culture in a sterile state. This needle may be a dull discard from the laboratory.

Trypticase soy agar and brain-heart infusion broth are used. Considerable trouble with the agar slants loosening from the side walls of the bottle and slipping down into the broth was experienced early in the studies. This can be prevented by etching several cross lines on one inner surface of the bottle, producing a roughened area which, with the angle of the glass, prevents slippage of the agar. It was found that etching with

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hydrofluoric acid was much more convenient than the dental drill method of Marvin.[2] First the surface is coated with paraffin; next lines are scratched through the paraffin with a stiff wire bent at 90° angle at the tip, then concentrated hydrofluoric acid is poured onto this area and allowed to remain in contact with the exposed glass surface for about 30 minutes. The bottles are then emptied and rinsed with water. A few minutes in a boiling water bath will melt the paraffin.

Further insurance against slippage can be obtained by making the agar slant one to one and one-half inches wide at the bottom. The agar medium is dispensed in the melted state (45-55°) C in amounts of approximately 50 ml. into each of the clean nursing bottles. The rubber caps are then applied to the bottle tops and the bottle caps are either punctured with the needle airway or, are arranged as previously stated.

Brain heart infusion broth is made up in a one liter Erlenmeyer flask, adapted to serve as a dispensing flask (Fig. 1). At the exterior of the airway tube a four inch length of rubber tubing terminating in a sterile cotton filter is attached. The bulb of a broken 5.0 ml. quantitative pipette filled with cotton is excellent for this purpose. Broken serological pipettes are used for the airway tube and a pinch-cock on the rubber is closed during autoclaving to prevent media boiling out of the tube during sterilization or moistening the cotton. The sterilized broth is dispensed aseptically by means of a length of rubber tubing which has a pinch-cock for controlling the flow of liquid. The tubing terminates in a small bore syringe needle which is enclosed in a tube with cotton around the upper rim for sterilization (Fig. 1). All media and equipment are autoclaved 121° C for 15 minutes. The bottles containing the agar are slanted so that the agar covers about half of the bottom diameter of the bottle (Fig. 2), and the upper edge of the slant approaches the neck. The sterile dispensing bottle is now inverted in a ring stand, the end of the airway is secured above the fluid level and the pinch-cock on the tube is opened. The protecting tube is removed from the dispensing needle and 50 ml. of the broth is added aseptically to each bottle containing an agar slant by puncturing the cap with the needle through an alcohol sponge. The partial vacuum of the bottles when no airway is used will just permit the addition of this amount. All bottles are incubated at 37° C for 48 hours to insure sterility. The bottles are then ready for use and are maintained at room temperature. We also use a second 4 oz. bottle with 100 ml. of liquid thioglycollate media for the detection of anerobes.

Fig. 1

Procedure

Blood for culturing is usually withdrawn from one of the prominent veins of the arm, though other sites can be used. The skin over the puncture site should be washed with green soap and water and covered with tincture of merthiolate or its equivalent and allowed to stand a few minutes before puncture to allow the disinfectant time to act. With all sterilization procedures there is a time element and if this part of the technique is done carelessly or hastily contaminants may be obtained. It is well to release the tourniquet before leaving the vein. This reduces the pressure and the needle can be pulled clear of the vein before the wound is covered with a sterile sponge, consequently the needle has no contact with the sponge of disinfectant. Twenty ml. of blood are contained in the sterile syringe; the rubber cap of an agar slant bottle is punctured with the needle

Fig. II

and 10 ml. of the blood is added to the broth in the bottle. The syringe is withdrawn and the remaining 10 ml. of blood added to a second smaller bottle of 100 ml. of thioglycollate media[3] for the detection of anerobes. Good technique, of course, demands that the needle be flamed between transfers though this actually may be superfluous if the bottle caps are kept moistened with merthiolate. In each case adequate sampling (10 ml. of blood) has been obtained and the quantity of media used is sufficient to dilute out the antibacterial properties of the blood or its antibodies.

The bottles are then shaken and labeled and if required para-aminobenzoic acid or pencillinase is added aseptically. They are then incubated at 37° and each bottle is inspected daily for growth. At 24 hours the agar slant bottle, if negative, is tilted so that the fluid washes up upon the agar slant. The cap is then pierced with the small sterile needle airway and the bottle is placed in an atmosphere of 10 percent carbon dioxide. Growth, when it occurs, is indicated by visible colonies on the agar slant. Turbidity of the broth or a pellicle may also be present. When growth occurs, the organism should be identified as rapidly as possible. Both bottles are maintained for thirty days before a final negative report is made.

Discussion

A method of blood culture technique has been presented. Contaminants have been very few, especially in the light of evidence[4,5] that bacteria are certainly circulating in the blood streams of normal people and on the surface of the skin. Dunham[6] reports that 34 percent of new born babies have positive blood cultures. The organism in these studies have usually been strains with little if any pathogenicity. It has also been demonstrated that hard chewing when mouth infections are present can produce positive blood cultures.[7,8] It is also well established that the blood stream harbors organisms after tooth extraction.[8,9,10] Pathogens in the blood usually mean that they also are in the tissues.

The physician is more interested in the antibiotic resistance or sensitivity of an organism than in its ultimate identification. Because of this shift in emphasis the number of organisms per ml. is no longer as important as it used to be. This determination has been eliminated from this technique. The examination for anerobes[11] should not be discarded as these may be isolated from the blood before their presence is suspected in the tissues.

Summary

A technique is presented for blood culture that utilizes materials available in any laboratory. The media employed will support most of the very fastidious micro-organisms. It is simple, inexpensive, and well adapted to routine use.

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Annual Convention ASMT

HEADQUARTERS: BROWN HOTEL
LOUISVILLE, KENTUCKY

June 14-18, 1953

See Advertising

Pages for

Convention Reservations

THE MEDICAL TECHNOLOGIST AND CIVIL DEFENSE

According to a recent letter from John B. Alsever, M.D., Chief, Blood Program, Defense Division, Federal Civil Defense Administration, the responsibility for organizing and training local civil defense units has been placed, by Congress, as a responsibility of each State government. Except for general plans and recommendations, the Federal government will not propose a national organized effort or plan for the use of medical technologists. State and county civil defense committees are meeting periodically to try to formulate coordinated workable programs.

According to the government bulletin, AG-11-1, in time of disaster all but the most essential laboratory activities would be curtailed. For the most part these essential tests, according to the bulletin, would be simple screening tests, i.e., simple tests for diabetes, hemoglobin determinations, hematocrit determinations and blood counts in case of radiation sickness. However, one type of laboratory service indispensable for large numbers of patients, would be blood bank procedures.

Many of our state societies are anxious to know what they can do in the civil defense program. The problems faced by rural and urban communities are individual. Large cities would face the problem of evacuation to suburban areas. Rural communities would supply and care for evacuees.

Programs can be instituted for blood grouping and Rh typing in rural communities which will be the sources of supply for whole blood in case of disaster to urban areas. A blood grouping program can be carried out inexpensively and with reasonable accuracy if simple blood grouping is done and the more complicated procedures are omitted. Identification cards can be issued and a file kept in some central office (see example)* Many industrial plants will make their own file copies and pay for the work.

If a program of this sort is considered, it must be done under professional supervision or certificate of approval from your State Department of Health Co-ordinating Laboratory and approval of the local civil defense committee.

The following suggestions are offered to state societies of medical technologists:

1. Contact your state and county civil defense committees and medical societies.
2. Cooperate with them in any program they may have instituted.
3. Be prepared to do blood groupings and Rh typings.
4. Appoint a civil defense chairman in state and district societies who will keep informed about civil defense activities in their localities.
5. Have available for disaster call the names, addresses and telephone numbers of all registered medical technologists, both active and inactive. Your membership chairmen would help in this work.
6. Promote the issuance of cards by hospitals and blood banks showing the blood group and Rh type of donors and patients.

*CIVILIAN DEFENSE BLOOD PROGRAM

Name _____

Address _____

City _____ State _____

Tested _____
Date

(Rh test with **BLOOD GROUP** _____

Anti-D only) **Rh TYPE** _____

District No. 2 Civil Defense Committee
Licensed by the State Department of Public Health
Doris S. Whitney, B.S., M.T., (ASCP).

EDITORIAL

Nearly forty years ago the author of this editorial graduated in medicine. At that time laboratory medicine was in its infancy. Aside from tissue pathology, clinical pathology consisted of rather simple laboratory procedures. The complement fixation test was just being initiated in the diagnosis of syphilis. Blood chemistry was yet to come, as were the basal metabolism test, blood typing, electrocardiography and a host of other tests that are now considered as part of a complete medical examination.

The acceptance of laboratory medicine by the profession at large was gradual. Some of us thought painfully gradual. It was not unusual for a clinical pathologist to stress the importance of his field to his colleagues in papers, before Medical Societies, as well as by pamphlets and paid space in Medical Journals.

Today clinical pathology has full recognition as a specialty in medicine. Clinical pathology is available practically to every city and hamlet in our nation. Thanks to the corps of well trained clinical pathologists and medical technologists who have worked hand in hand to bring about this achievement.

With this achievement certain tendencies have developed that are not altogether to the best interest of medicine. While laboratory medicine is important, it was never intended to minimize clinical medicine. History taking in detail and a complete physical examination are still as important today as they always have been. A complement fixation test by itself cannot give the diagnosis of syphilis, nor can an agglutination test make a diagnosis of typhoid fever or undulant fever, important as these tests are. So it is with various other laboratory procedures. These facts have been stressed through the years in text books on clinical pathology, and by all reputable workers in this field.

It is therefore still of the utmost importance to emphasize in our teaching curricula for internes, residents, and medical technologists that the laboratory cannot be used as a short-cut in establishing a diagnosis, but only in its rightful place as a valuable adjunct. With this training, inevitably recruits in medicine will develop who will have a sense of appreciation and who can evaluate what help the laboratory can give and what it cannot give in the field of diagnosis, a fact that sometimes seems to be forgotten and, for that reason, must be re-emphasized from time to time.

A. H. BRADEN, M.D.

* * * *

Send all changes of name and/or address promptly to: ASMT, 25 Hermann Professional Bldg., Houston 25, Texas, AND TO: The Registry of Medical Technologists, Muncie, Indiana.

THE GAVEL

Greetings to each of you from the office of the President! To those of you who have just joined our ranks, may I extend a warm welcome. To those of you who have been members previous to January, 1953, may I express my THANKS for your patient understanding of the responsibilities of this office, your loyalty to ASMT, its Officers and Board of Directors, for your devotion to duty, and for the cooperation that you have given to the Executive Office, the Officers and Board of Directors, and the Committees of ASMT throughout the year.

Because of this cooperation in the past we have been able to accomplish many things and are looking for more to come with:

1. Officer ratings for the MT(ASCP) going into the Armed Forces.
2. Representation on the Board of Registry (ASCP).
3. Representation on the Board of Approved Schools of Medical Technology (ASCP).
4. Representation on ASCP Committee to study Reclassification and Training of Medical Technologists.
5. Representation on ASCP Film committee—this film to be used for Recruitment of students.
6. Glassner & Associates of Chicago retained as Public Relations counsel.
7. Higher Civil Service ratings within reach. A final hearing is to be given before June 1953.
8. An intensified Recruitment Program in progress.
9. Two ASMT members invited to speak in Chicago at the hearing on the Crane proposal on Reclassification and Training of Medical Technologists.
10. ASMT represented at the Health Information Foundation meeting and at the UNESCO meetings.
11. The AMERICAN JOURNAL OF MEDICAL TECHNOLOGY with an Editorial Board composed of Specialists.
12. The President of ASMT and the President of ASCP cosigning a letter to all Pathologists stressing the need of closer cooperation between the Pathologists and the Medical Technologists—especially asking the Pathologists to open an approved school.

These are but a few of the things we have done with your help.

In order to finish the year with the same degree of success that we have enjoyed thus far, I ask your cooperation on the following:

1. The Gellis questionnaire: Fill this out and return immediately. This is important not only to Dr. Gellis, but to ASMT.
2. The Standards and Studies Committee questionnaire: Answer each section and return immediately. This survey is most important for general information.
3. Membership: Every member get a member. If you will resolve to get just one new ASMT member, and DO IT—the Membership committee will be very happy, so much we can accomplish with an increased membership. The time to get these new members is to contact them just as soon as they become registered. Start with those in your own Laboratory.
4. Recruitment: There are many opportunities for recruitment of students for the A.M.A. Approved Schools. Take advantage of every opportunity. The Recruitment Committee has a wonderful set of slides on Medical Technology that will help you in your talks to groups of prospective students. Talk, talk, and talk some more. We can never recruit too many students! Your personal appearance, enthusiasm, and appeal are your best tools.

5. Constitution and By-Laws: The proposed revision appears in this issue. Study it carefully, discuss it in detail at your State meeting, decide what you want. If there is anything that you want to add, be prepared to submit it at the June meeting of the House of Delegates.
6. Nominations and Elections: A finer array of capable candidates has never been presented. Study this slate carefully—then elect those who will be your choice. The committee has carefully screened each not only for ability but for loyalty to ASMT. To be fair to all our affiliated societies, they have been chosen with consideration of their geographical distribution.
7. State Officers and Committee members: Save the ASMT Newsletters and pass them on with your file to your successor.
8. Legislation: Be sure to report any activity or possible activity to the ASMT Legislation Chairman. This information is necessary for possible action as well as for the record.
9. 1953 ASMT Convention: A wonderful program has been arranged—both scientifically and socially. Your attendance is urgently requested. This has been planned for YOU. YOU will be the loser if you do not come.
10. Registry of Medical Technologists (ASCP): Plan to visit the Registry. This is an opportunity to see your Registry in action, one not to be missed.

Thanks to each of you for making this year a success. Your ideas have been valuable, your opinions respected and appreciated, and your cooperation gratefully received.

Sadie Cartwright, MT(ASCP)

AMONG THE NEW BOOKS

SYNOVIAL FLUID CHANGES IN JOINT DISEASE: by Marian W. Ropes, M.D., Associate Physician, Massachusetts General Hospital, Assistant Clinical Professor of Medicine Harvard Medical School, and Walter Bauer, M.D., Chief of Medical Services, Massachusetts General Hospital, Jackson Professor of Clinical Medicine and Director of Robert W. Lovett Memorial Foundation for the Study of Crippling Disease, Harvard Medical School. Harvard University Press, Boston, Mass 1953. 143 pages, 15 illustrations, 14 tables, Chart. $4.00.

The most complete study of its subject presented primarily for the physician or research worker, this book has information for the medical technologist which he does not find in other texts. Beside the normal findings for synovial fluid, there are comparative values given in the various forms of joint disease. Although techniques are not discussed here, the very complete bibliography has such references as will prove invaluable in the laboratory work required.

PRACTICAL CLINICAL CHEMISTRY, a guide for Technicians: by Alma Hiller, Ph.D. Associate Attending Biochemist in Charge of Clinical Chemistry, the Presbyterian Hospital, Chicago. Charles C. Thomas, Springfield, Ill., 1953. 279 pages, 13 tables, 50 pages of outlines of methods for desk use. $6.50.

Each determination is given with complete directions. Where special apparatus is necessary this is described in detail. Reagents and procedures, with calculations, are given for determinations in blood, urine, and other body fluids. The outlines of procedures are given in the text, and are repeated on perforated pages which may easily be removed for desk use. The suggestion is that they be placed under cellophane and used in this manner. The book is most practical for general routine work.

THE PHARMACOLOGY OF ANESTHETIC DRUGS: John Adriani, M.D. Director, Department of Anesthesiology, Charity Hospital, New Orleans, Louisiana. Ed. 3. Charles C. Thomas, Springfield, Ill., 1952. 179 pages, illustrated by diagrams. $9.50.

For the medical technologist employed also as anesthetist in the small hospital this volume would prove of value. It is, as the title indicates, of primary interest to the nurse, medical student or physician engaged in this branch of medicine.

REPORT—TO THE MEMBERSHIP OF THE ASMT

In June, 1949, it was deemed necessary to increase the facilities of the Executive Office—The Board of Directors ordered a full time Office set-up and obtained the services of a full-time executive secretary and editor of the AJMT—said publication to be edited from the same office. This was a new venture and it was agreed that it would be on a trial basis for a period of two years.

In June, 1950—The chairman of the Board asked all Board members to submit a report re: opinion on operation of the set-up as stated above and also as compared with the set-up of other organizations. This was done and each member contributed a report according to the information he was able to secure. As a result there appeared a block NOTICE in two issues of the AJMT. The notice appeared in the January-February issue of 1951, page 38; and the March-April, 1951, issue, page 102, and was thus worded:

NOTICE

"In June, 1949, it was deemed necessary to increase the facilities of our Executive Office. At that time the Board of Directors ordered a full-time Office set-up and obtained the services of a full-time Secretary-Editor, the AJMT to be edited from the same office. Since this was a new venture, the Board felt it wise to specify that such arrangement to be for a period of two years. Next June, that period will be over. This present Board has investigated the set-up of comparable offices in other organizations and has agreed to recommend that our present arrangement be continued. If that Recommendation is approved by the new Board, it will be necessary to simultaneously appoint an Executive Secretary-Editor. All applications for this position should be sent to the Chairman of the Board of Directors for 1951-52, Miss Lavina White, M.T. (ASCP), Pueblo Clinic, Pueblo, Colorado, and should be in her hands not later than May 1, 1951."

Therefore, with the above reports in mind, the Notice in the Journal to the membership that our set-up has proved to be a satisfactory one, along with the recent report of C. Patton Steele—as a member of the investigation committee re: possible location for an Executive Office, who gives us a report on the things that we already have at our disposal and to our advantage, it seems most unnecessary, from the standpoint of advantages to be received by ASMT as well as being quite a financial burden for ASMT, to consider a move of the Executive Office or a change in the policy of the American Journal of Medical Technology. At any rate—sufficient reasons have not been stated. Respectfully submitted,

Lucile Harris, M.T. (ASCP), Member—Board of Directors, ASMT
c/o Drs. Sellers, Adamson and Smith, Abilene, Texas

ANNOUNCEMENTS

Conventions: Canadian Society of Laboratory Technologists: May 17-20, 1953, Edmonton, Alberta. The Hotel Macdonald headquarters. All members of A.S.M.T. are cordially invited.

The 30th annual Conference of the American Physical Therapy Association will be held at the Baker Hotel, Dallas, Texas, June 15-19, 1953. The theme for the program will be the Patient—His Evaluation and Program for Rehabilitation.

CONSTITUTION AND BY-LAWS

The following amendment to the Constitution was inadvertently omitted from the report in the September issue of the Journal: ARTICLE IX. OFFICERS, DIRECTORS, ADVISORY COUNCIL, Section 3. "The Advisory Council shall be composed of the Board of Directors of this Society, the President and immediate Past President of each subordinate society. The duties of the Advisory Council shall be to consider and promote the interests of the Society."

REPORT OF THE COMMITTEE ON NOMINATIONS AND ELECTIONS

The following members of the American Society of Medical Technologists will be presented for the vote of the House of Delegates at its annual meeting on June 16, 1953, in Louisville, Kentucky.

President-elect (one to be elected)

Ruth Hovde, 515 Delaware Ave., S. E., Apt. 104, Minneapolis, Minn. Ruth graduated from the University of Minnesota with a B.S. in Medical Technology in 1938, and with an M.S. in Hematology in 1949. She is now an Instructor and Senior Advisor in Medical Technology in charge of the curriculum for the Course in Medical Technology at the University of Minnesota. She has been a Member of ASMT since 1946; President Minnesota Society of Medical Technologists 1951-52; Member of Board of Directors of the Twin Cities' Society of Medical Technologists 1950-53; Member of the Board of Directors of Minnesota Society of Medical Technologists 1950-53; Advisory Council ASMT 1951-52; Delegate ASMT conventions 1950-51-52; Representative for ASMT to Health Information Foundation 1952-53; Member ASMT Public Relations Committee 1952-53; Representative for ASMT on the ASCP Film committee 1952-53; Representative for ASMT at the ASCP Executive committee meeting on the Crane Proposal 1952.

Wade Marsh, Jr., The Baroness Erlanger Hospital, Chattanooga, Tenn. Wade graduated from the University of Kentucky with a B.S. in Medical Technology in 1948. He was at the Good Samaritan Hospital, Lexington, Ky. in 1948-49, and was Chief Medical Technologist at the Passovant Memorial Hospital in Jacksonville, Illinois in 1949. He has been Chief Medical Technologist since 1950 at the Baroness Erlanger Hospital, the Tennessee Tuberculosis Hospital, and Childrens Hospital in Chattanooga, Tennessee. Wade has been a member of ASMT since 1948; Vice-President of Kentucky SMT 1949-50; Treasurer of Tennessee SMT 1951-52; President Tennessee SMT 1952-53; Delegate to ASMT Convention (from Ky.) 1949, (from Tenn.) 1950-51; Member ASMT Legislation Committee 1950-51; Chairman ASMT Legislation Committee 1951-52.

Secretary (one to be elected)

Sister Mary Simeonette Savage, 851 S. 4th St., Louisville, Ky. Sister received her A.B. Degree from Nazareth College, Louisville, Ky. with majors in chemistry and biology; and her M.S. from Catholic University of America, Washington, D. C., with a major in bacteriology, supported by mycology, physiology and parasitology. She has also taken courses under Dr. Diamond and Dr. Papanicolaou, and for the last twelve years has been teaching the Medical Technology students of St. Joseph Infirmary. Sister Simeonette is registered as a Bacteriology Specialist (ASCP) and has been a member of ASMT since 1949; Member of Constitutional Committee which drafted the Louisville District Society Constitution 1949; Vice-President of the Louisville Society of Medical Technologists 1949; Member of Program Committee LSMT 1949-50-51; Member of Constitution Committee of the Kentucky SSMT which drafted revised KSSMT Constitution; Chairman of Mycology Seminar Committee, KSSMT 1949; President of KSSMT 1951; Chairman of KSSMT Public Relations and Vocational Guidance Committees 1952; Won first award (ASMT) in 1949 for paper on biochemistry; Delegate to ASMT Conventions 1949-50-51-52; Chairman of ASMT Program Committee 1953.

Violetta Wakefield, 1209 N. 34th St., Fort Smith, Ark. Vi took her medical technology training in Fort Smith under Dr. A. A. Blair, and is this year celebrating her 30th year at the Cooper Clinic. Dr. Blair, a member of the Board of Internal Medicine and one time Governor for

the FACP for the State of Arkansas, is still her boss. She has been a Member of ASMT since 1939; a Charter member of Arkansas SMT 1938; President Arkansas SMT 1938-39, 1947-48; Member of Board of Directors Arkansas SMT 1939-40-41, 1950-52; Chairman Arkansas SMT Legislation Committee 1948-50; Chairman Arkansas SMT Constitution and By-Laws Committee 1951-53; Member ASMT Legislation Committee 1947-48; Member ASMT Nominations and Elections Committee 1949-50; Chairman ASMT Nominations and Elections Committee 1950-51, 1951-52; Delegate to ASMT Conventions 1939, '41, '47, '48, '49, '51, and '52.

Board of Directors (two to be elected)

Leanor D. Haley, 310 Cedar St., New Haven, Conn. Leanor graduated from Duke University with a B.A. (Pre-medical) in 1942; Completed her training in the School of Medical Technology, University of Virginia School of Medicine in 1944; Passed her National Board Examination in April 1944; was Senior Technologist in the Department Clinical Pathology, University of Virginia School of Medicine 1944-45; was Chief Bacteriologist, Biological Division, John Hopkins Hospital 1945-46; received her M.A. in 1947, and her Ph.D. in 1949 (Medical bacteriology and mycology) from Duke University. She was Consultant for clinical pathology laboratory, Lincoln Hospital, Durham, N. C. 1948-49; a Graduate Assistant in the Department of Bacteriology, Duke University School of Medicine 1947-49; an Instructor at Yale University School of Medicine, Department of Microbiology 1949-50; Assistant Professor at Yale University School of Medicine, Department of Microbiology 1950-...; and Director of Microbiological Laboratories at Grace—New Haven Hospitals 1952-... Leanor has been a member of ASMT since 1950; Chairman of Research Committee of Connecticut Society of Medical Technologists 1950-51; Member Research Committee of Conn. Society of Medical Technologists 1952-...; Winner of Registry Award 1951; President-elect Conn. Society of Medical Technologists 1951; and President of Conn. Society of Medical Technologists 1952-53.

Ellen Skirmont, 338 S. East Ave., Oak Park, Ill. Ellen has been a Registered Medical Technologist for thirteen years, and is Chief Medical Technologist for Argonne National Laboratory, one of the Atomic Energy Labs; was one of the first Medical Technologists at Oak Ridge, Tennessee. She has been a Member of ASMT since 1946; Delegate ASMT Convention 1947-48-49-50-51-52; Presented Papers ASMT Convention 1947-48-49-50-51; Member ASMT Convention Program Committee 1951; Presiding Officer of Scientific Session ASMT 1950; President-elect and President IAMT 1948-50; Member ASMT Public Relations Committee 1951; Chairman ASMT Public Relations Committee 1951-52-; ASMT Representative for American Hospital Association; President Alpha Mu Tau 1951-52-53; Editor of **Lab Page** in **Hospital Topics** Magazine.

John N. Frazer, St. Joseph's Hospital, Thibodaux, La. Johnny had his pre-medical training at Millsaps College, Jackson, Mississippi. His two years in Medical Technology were in the Mercy Hospital and Street Clinic, Jackson. He joined ASMT in 1941. Past president of Mississippi SMT, charter member. Presented Third Award paper at Roanoke Convention 1949; several short papers published in AJMT; charter member Mississippi SMT; served on Program and Paper Awards Committees; ASMT Legislative Committee 1952-53; delegate ASMT conventions 1949-50.

Oscar Stewart, 2032 E. 14th St., Tulsa, Oklahoma. Oscar attended Oklahoma University and Colorado A. & M. College, and has had fifteen years experience in laboratory work. He has been a Member of ASMT since 1942; President of Tulsa SMT 1944; President Oklahoma SMT 1945; Delegate ASMT Convention 1945; Chairman Commercial Exhibits Com-

mittee ASMT 1946; Chairman Constitution and By-Laws Committee ASMT 1946-47; Member Finance Committee ASMT 1947-49; and Member of Board of Directors ASMT 1947-50.

NATIONAL LABORATORY OPEN HOUSE

For 100 percent ASMT MEMBERSHIP participation in the National Laboratory Open House for 1953, during National Hospital Week, contact your state society Recruitment and Public Relations Chairmen for details. Your hospital administrator will also have information. Write to A.S.M.T., Suite 25, Hermann Professional Bldg., Houston 25, Texas, for the outline prepared by the Public Relations Committee.

CONVENTION ANNOUNCEMENTS

Dr. Malcolm L. Barnes, president of the Kentucky Society of Pathologists, will be the Honorary Chairman for the 21st Annual Convention of ASMT to be held at the Brown Hotel, Louisville, Kentucky, June 14-18, 1953. Dr. Barnes, a very busy man, has had a ready ear to all our plans and has been brimful of ideas to make this convention outstanding. To him we give our thanks and appreciation.

The convention begins with registration on Sunday, June 14th at 8 a.m., in the lobby on the second floor. The hostesses will be there to direct you to your church. The tour of the city on Sunday afternoon will be made by as many private cars as possible. At the reception Sunday evening in the Bluegrass Room you will be the guests of the Kentucky State Society of Medical Technologists.

Monday evening there will be passes for the harness races (of course there will be an admission tax). Bus fare to the races $0.50.

Tuesday, there will be a tour of Louisville industries for those not attending the House of Delegates meeting. Tour cost $1.75. The House of Delegates luncheon—$2.50.

Wednesday evening—a buffet supper. ASMT committees at special tables. Stephen Foster melodies—$3.00.

Thursday afternoon—trip to Bardstown and Gethesemane. "My Old Kentucky Home" and the Old St. Joseph Cathedral—$2.50.

Thursday evening—the annual banquet. Convention Awards. Special table—Past-Presidents of ASMT. Special table—Presidents of State Societies. Guest speaker—Dr. O. O. Miller—Australian born—noted Kentucky speaker. (Formal dress—but come informal if you wish)—$7.00.

Note: Prices may change. The above are tentative. Bring your A.S.M.T. Card for identification.

THE PROGRAM COMMITTEE REPORTS

The Program Committee has been working for several months trying to procure experts in the widely diversified fields of clinical laboratory medicine so that the program will come up to your expectations. We feel that this meeting of the ASMT will offer unexcelled opportunities to the members of our society to bring themselves up-to-date on many of the problems of the profession. The scientific program is broad in scope and practical in emphasis.

From time to time, the Officers of ASMT have been told by members who are sincerely interested in the society that they do not understand how the American Society of Medical Technologists works. Since many of our members feel this way, this year your Board of Directors asked the Program Committee to highlight the Professional Workshops. We are happy to announce to you that each of the standing Committees of ASMT will present a Workshop to illustrate the activities of the Committee and how it contributes to the over-all functioning of ASMT. It is only when *all* our members are well informed about *all* the aspects of medical technologists and their professional societies that we can hope for a harmonius integration of state and national organizations.

Public Relations is a much-discussed topic these days. There are a great many opinions about how public relations of any organization should be conducted and who should conduct them. The Public Relations Committee under the leadership of Ellen Skirmont will try to answer these questions for us.

We all know that the shortage of medical technologists has reached the acute stage. In order to staff our medical laboratories, it will be necessary to lower our standards if we do not bring new recruits into the field. Sister Barbara Clare and the Recruitment Committee with the able assistance of Mrs. Ruth Drummond will give us new suggestions for making medical technology attractive to high school and college students. Education and Research, Constitution and By-Laws, Membership, Standards and Studies, Legislation, Nominations and Elections, Service Fund and Finance, all our Committees, are preparing to make Workshop Day, Monday of Convention week, a very important one this year.

Among the guest speakers whom we did not tell you about in the last issue of the Journal are: Dr. Lall Montgomery of the Board of Registry, Dr. Clayton Loosli of the University of Chicago, Dr. George Shinowara of Ohio State University, Dr. Raphael Isaacs of Chicago, Dr. William Lotspeich of the University of Cincinnati, Dr. Edna Sobel of Cincinatti, Dr. M. H. Grossman of Chattanooga, Tenn., Dr. Louis Smith of Dallas, Texas, and several guest speakers from Kentucky.

Our own medical technologists will make many valuable contributions to the program: Helen Madden, of Boston, Jane Frances Taylor of Columbus, Ohio, and Jane Haber of New York, will present a Round Table Discussion of the latest techniques in Rh Blood Bank work. Nancy Lu Conrad of Columbus, Ohio, will report on a new culture medium which she has developed. Berenice Koster of St. Louis, will give a paper on blood volume studies. Dr. Leonor Haley of Connecticut, will discuss the mycological diagnosis of the dermatophytes. Sister Mary Carmelite, RSM, of Missouri, will tell us how good public relations can be maintained in a small hospital between the nursing staff and the medical technologists. Sister Mary Antonia, S.C.N., will give a paper reporting her research on a new anticoagulant. Anne Sommer will tell us about a newly developed substrate for acid and alkaline phosphatase tests. Mrs. Aileen Burge Wright will read a paper on the results of her work in enteric bacteriology. Mildred Talluto will present a paper on blood amylase studies. Sister Dolorosa, S.S.M., and Dorothy Nary who have not submitted their subjects to the Program Committee at this time will present papers also. The May issue of the Journal will carry the entire program.

The scientific program will take place on Wednesday and Thursday of Convention week. We hope that all of you will be in Louisville for the 1953 ASMT Convention.

Your Program Committee,
Nila Maze
Rachel Lehman
Sarah Hanna
Ruth Bluemlein
Ed. Crowe
Sister Mary Simeonette Savage,
Chairman

For more convention announcements see advertising pages.

THE BROWN HOTEL, LOUISVILLE, KY.

NOTICE TO THE SISTERS

Your Convention Hospitality Committee is anxious to hear from all Sisters who wish housing in Louisville Convents. Let us know by the first week in April.

Sister M. Christina Wedding,
St. Mary's and Elizabeth's Hospital,
Louisville 10, Kentucky.

OPEN HOUSE AT THE REGISTRY OFFICE

Lavina White says: "The OPEN HOUSE at the Board of Registry will be a fitting occasion for all the members of A.S.M.T. to stop over at Muncie where we are offered the most delightful hospitality by Dr. Montgomery, Mrs. Drummond and all the staff. This will be the most opportune time to drop in and see the Registry in action: to see how the details of your own professional status are maintained. Your original application, with all the details you labored over to prove your qualifications, and all the followups of personal problems since, will make you feel close to your Registry. The whole office staff is vitally interested in Jane Doe's chemistry credits, especially if her alma mater has changed the listing of courses. They go over many catalogues to correlate her academic prerequisites. The question of foreign credits is something of a Herculean task to align with our American college requirements. Again someone forgets to send a new address with the renewal and writes frantically for the annual seal so the new hospital or clinic will be sure she is REGISTERED. It takes the utmost patience to cover all these points and more, and the Registry is doing it for us. Let's take advantage of the location of the convention and meet in Muncie, Indiana, on the way to, or from Louisville. You'll agree that it is a wonderful chance to meet the Registry gang and learn of their sincere interest in us and our profession."

Mrs. Drummond Says:

Here is what we would like to do for you while you are in Muncie:

Show you the Registry office.
Give you a bite to eat.
Put you up for the night if you like.
Offer you bus transportation to or from Louisville, if you need it.

In order to do these things, we will need your cooperation at once, so won't you please fill out the coupon below, and send it in to us right away, before you forget about it, if you are going to stop off?

Cut out this coupon and mail to:

Registry of Medical Technologists
700 South Council Street
Muncie, Indiana

I plan to visit the Registry office

June 13........

June 19........

I will arrive in time for lunch.....

I will need hotel accommodations for the night.....

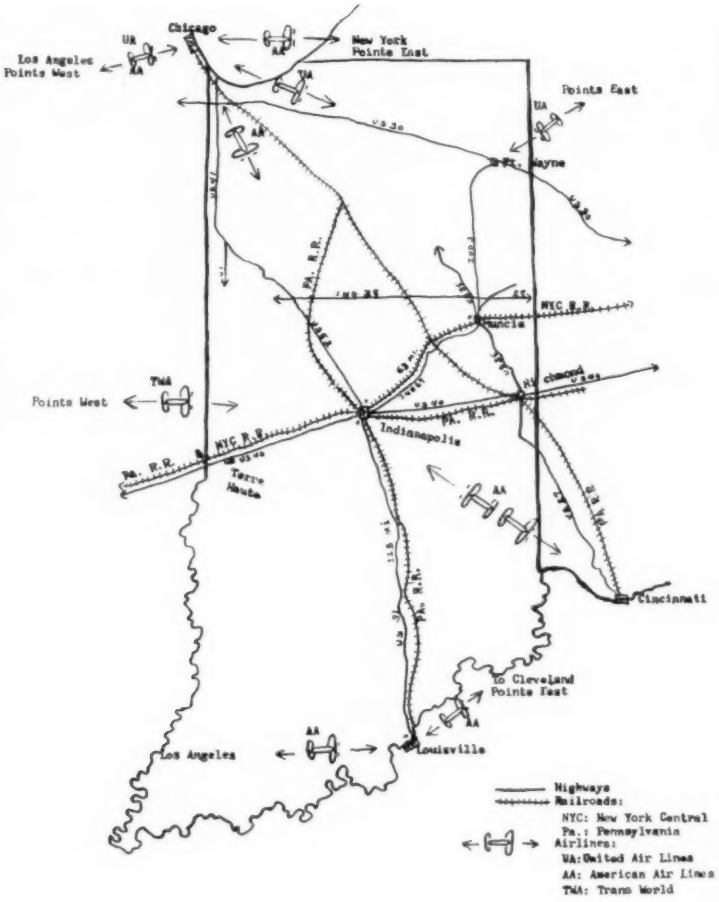
I will need bus accommodations to Louisville.....

I will need bus accommodations from Louisville.....

Signed..

Address..

PLEASE RETURN BY MAY 1



Saturday, June 13 or Friday, June 19

Here is a map which shows how you can get to Muncie by air, train, bus or car. We hope that many of you will stop to see the Registry office either on your way to the ASMT meeting in Louisville, or on your way home from there.

REPORT OF COMMITTEE ON CONSTITUTION AND BY-LAWS

The Committee on Constitution and By-Laws of the American Society of Medical Technologists, in accordance with the instructions of the House of Delegates' meeting of June 23, 1952, has prepared the following Revision of the By-Laws of this Society and will submit such proposed Revision at the 1953 Annual Meeting of the House of Delegates.

The following amendments to the Articles of Incorporation of this Society which have been approved by more than two-thirds of the members of the Board of Directors of this Society will also be submitted to the House of Delegates and to the members of this Society at the same Annual Meeting.

Respectfully submitted,
Mary Ann Johnson (New Jersey)
Margaret Haraway (Oklahoma)
John Mooty (Wisconsin)
M. Claire Murray (North Dakota)
Charlotte Thumm (Ohio)
Rose M. Hackman, Chairman (Colorado)

March 1, 1953

Proposed Amendments to Articles II, VIII, IX, X, XI, XII, and XIII of the Articles of Incorporation of the American Society of Medical Technologists

ARTICLE II
PURPOSES

The purposes of this corporation are:

To promote higher standards in clinical laboratory methods and research.

To elevate the status of those specializing in medical laboratory technique.

To create mutual understanding and cooperation between the medical technologist and physicians and all others who are employed in the interest of individual or public health.

To issue charters to constituent societies.

To promote the mutual aid and benefits of its members.

In general to do anything and everything necessary and proper to the conduct of a society of this nature, and for the purpose of attaining or furthering any of its objects to do any and all other acts and things, and to exercise any and all other powers which now or hereafter may be authorized by law.

ARTICLE VIII
MEMBERSHIP

The membership of this Society shall consist of such classes and subclasses as may be provided by the By-Laws of this Society, and the members in each of such classes and subclasses shall have such rights and privileges and shall be governed by such rules of eligibility, admission, retention and dismissal as may be prescribed by the By-Laws.

ARTICLE IX
OFFICERS, DIRECTORS, ADVISORY COUNCIL

Section 1. The officers of this Society shall be: a President, a President-Elect, a Recording Secretary, and a Treasurer, and such other officers as may be provided by the By-Laws of this Society. The respective terms of office and duties of all of such officers shall be as prescribed by the By-Laws.

Section 2. The Board of Directors of this Society shall consist of the President, the immediate Past-President, the President-Elect, the Recording Secretary and the Treasurer, during their respective terms of office, and in addition six members of the Society to be elected by the House of Delegates. The Board of Directors shall constitute the governing body of this Society while the House of Delegates is not in session.

Section 3. There shall also be an Advisory Council whose membership shall consist of the members of the Board of Directors of this Society and one representative from each constituent society who shall be the president or immediate past president of such society or an alternate as may be provided for by the By-Laws of this Society. The Advisory Council shall consider and promote the interests of this Society and shall perform such other duties as may be provided by the By-Laws.

ARTICLE X
HOUSE OF DELEGATES

Section 1. There shall be a House of Delegates whose membership shall consist of: (a) The members of the Board of Directors and the members of the Advisory Council or the respective alternates for any members of the Advisory Council; and (b) Delegates (or their alternates, as the By-Laws may provide) elected or designated by the constituent societies on a representative basis as provided for in the By-Laws of this Society.

Section 2. The duties of the House of Delegates shall be those of the governing body of this Society.

ARTICLE XI
CONSTITUENT SOCIETIES

Section 1. This Society shall be composed of the constituent societies which now hold, and which may hereafter be granted, charters from this Society. Each state, the District of Columbia and each territory, possession or dependency of the United States of America, and each foreign nation shall constitute a geographical unit for the chartering of a constituent society therein.

Section 2. Each Society which is now chartered by this Society as a subordinate society shall be duly recognized as a constituent society, and additional constituent societies may be formed and chartered in accordance with such requirements and procedure as may be provided by the By-Laws of this Society.

Section 3. The charter of any constituent society may be revoked or reinstated upon such grounds and conditions and in accordance with such procedure as may be prescribed by the By-Laws of this Society.

ARTICLE XII
BY-LAWS

The House of Delegates shall have the power to adopt, enact and amend By-Laws consistent with the Articles of Incorporation and the general objects and purposes of the Society and not in conflict with the laws of the State of Michigan.

ARTICLE XIII
AMENDMENTS

These Articles of Incorporation may be amended by a majority vote of the members of this Society entitled to voting privileges, provided that notice of the proposed amendment or amendments shall have been published in the Society's official organ at least sixty days and not more than one hundred twenty days in advance of the annual or special meeting at which such proposed amendment or amendments shall be submitted or shall have been mailed to the members of the Society having voting privileges at least sixty days and not more than one hundred twenty days prior to such meeting, and that the proposed amendment or amendments shall have been approved by a two-thirds majority vote of the Board of Directors within one year prior to such meeting.

Proposed Revision of the By-Laws of the American Society of Medical Technologists

ARTICLE I
NAME

The name of this corporation is American Society of Medical Technologists.

ARTICLE II
PURPOSES

The purposes of this society are to promote higher standards in clinical laboratory methods and research; to elevate the status of those specializing in medical laboratory technique; to create mutual understanding between the medical technologist and physicians and all others who are employed in the interest of individual or public health; to issue charters to constituent societies; to promote the mutual aid and benefits of its members; in general, to do anything and everything necessary and proper to the conduct of a society of this nature, and for the purpose of attaining or furthering any of its objects to do any and all other acts and things, and to exercise any and all other powers which now or hereafter may be authorized by law.

ARTICLE III
CONSTITUENT AND BRANCH SOCIETIES

Section 1. This Society shall be composed of constituent societies and of such branch societies as shall be organized and sponsored by the constituent societies.

Section 2. There may be one, and only one, constituent society in each geographically defined state, District of Columbia, territory, possession or dependency of the United States and in each foreign nation. Any number of branch societies may be organized by a constituent society within the boundary of its geographical area.

Section 3. The name of each constituent society shall contain the geographical location thereof and the words medical technologists, or the equivalent of these words. A constituent society may use the name of division of the American Society of Medical Technologists.

Section 4. A constituent society can be organized by three or more persons eligible for active membership in this Society. These persons shall petition for permission to organize a constituent society on a form supplied by the executive secretary which shall be submitted to the chairman of the Board of Directors and be approved by that Board. The petition shall be accompanied by an application fee of $5.00. When the petition has been approved, six copies of their proposed constitution and by-laws or, if the group wishes to become incorporated, six copies of their proposed articles of incorporation and by-laws shall be submitted to the constitution and by-laws committee of this society accompanied by a list of officers and charter members. The committee shall bring a report and a recommendation concerning the chartering of this group to the House of Delegates at the next Annual Session of the Society.

Section 5. A charter for the organization of a constituent society may be granted only by the House of Delegates.

Section 6. The duties and functions of a constituent society are defined as follows:
(a) It shall send to the president, the president-elect, the recording secretary and the executive secretary of this society the names and addresses of its officers not more than two weeks after their election.
(b) It shall send to the executive secretary within two weeks after members have paid dues a duplicate, typewritten, classified list of members who have paid dues, together with their addresses and registry numbers or degree statuses. Dues shall be submitted to the executive secretary within two weeks after they have been paid by a member.
(c) It shall sustain its status with this Society by maintaining a constitution and by-laws in harmony with the By-Laws of this Society and by maintaining adequate supervision over the branch societies which have been organized under its direction to insure that their constitutions and by-laws are kept in harmony with the By-Laws of this Society. The types of membership and qualifications for membership in a branch society shall be determined by the constituent society under which it is organized, except that all members of a branch society must abide by the Code of Ethics of this Society, and at least one-fourth of the members shall be active members in good standing of this Society, and the president of a branch society shall be an active member of this Society.
(d) It shall submit to the constitution and by-laws committee of this Society all proposed amendments to the constitution and by-laws of the constituent society at least sixty days before the approval of such committee is desired. Six copies of such amendments shall be submitted. When the proposed amendments have been approved by the Board of Directors of this Society they may be adopted. A constituent society shall report the adoption of any amendments to its constitution and by-laws to the constitution and by-laws committee of this Society within two weeks after their adoption.
(e) It shall send any proposed amendments to the By-Laws of this Society to the constitution and by-laws committee of this Society not later than December 1st and such amendments shall be presented to the House of Delegates at the next Annual Session. Six copies shall be submitted. Proposed amendments to the By-Laws of this Society may be submitted by a constituent society or by an active or life member of this Society.
(f) It shall send annually before September 15th to the executive secretary a charter fee of $1.00 which provides for the renewal of its charter. The renewal seal shall be affixed by the constituent society to its charter.
(g) It shall send promptly to the executive secretary information concerning the transfer of a member in or out of its geographical area.
(h) It shall send to the executive secretary at least two weeks before the opening of the Annual Session, and sooner when possible, a list of delegates and their alternates to the Annual Session. The addresses and registry numbers or degree statuses of the delegates and alternates shall be included.

Section 7. This Society shall be empowered to revoke the charter of a constituent society by majority vote of the House of Delegates provided the Board of Directors by a two-thirds vote favors the revocation of such charter and provided, further, that such constituent society shall have had at least thirty days notice of the charges filed against it and shall have had an opportunity to be heard and to defend itself before the House of Delegates. The charter of a constituent society may be revoked for any of the following causes:
(a) Failure of such society to require that all its members abide by the Code of Ethics of this Society.
(b) Adoption by the constituent society of policies or procedures contrary to the purposes of this Society.
(c) Failure of the constituent society to cooperate reasonably with this Society.

Section 8. Any constituent society whose charter has been revoked may be reinstated by a two-thirds vote of the House of Delegates upon application, provided such society is no longer in default and pays all expenses incurred, plus a reinstatement fee of five dollars ($5.00).

Section 9. Any constituent society shall revoke the charter of a branch society organized under its direction for failure of the branch society to comply with both the Code of Ethics of this Society and the By-Laws under which the branch society was organized and amendments thereto.

ARTICLE IV
MEMBERSHIP

Section 1. The membership of this Society shall consist of the following classes:
(a) Active Member. Any medical technologist who (1) is registered M.T. (ASCP) by the Registry of Medical Technologists of the American Society of Clinical Pathologists and is in good standing with that Registry, or (2) possesses a master's or higher degree in bacteriology, biochemistry, hematology, histology, parasitology, or serology from an accredited college, or (3) holds a limited certificate in chemistry or bacteriology from the Registry of Medical Technologists of the American Society of Clinical Pathologists, or (4) holds a limited certificate in histologic technique from the above mentioned Registry and has ninety or more semester credit hours from an accredited college of which at least twenty-seven hours are in science including chemistry and biology, with lecture and laboratory in each, shall be eligible for active membership in this Society provided, further, that he is an active member of the constituent society of the geographical area in which he is employed or resides if there be a constituent society in such area. An active member shall have all the rights and privileges of this Society.
(b) Affiliate Member. Any person who holds a certificate from and is in good standing with the Board of Registry of Medical Technologists of the American Society of Clinical Pathologists without meeting the requirements for active membership in this Society shall be eligible for affiliate membership in this Society provided that he is a member of the constituent society of the geographical area in which he is employed or resides if there be a constituent society in such area. An affiliate member is entitled to all the privileges of membership in this Society with the exception of voting, holding office, and serving on any Board or standing committee of this Society.
(c) Honorary Member. Any person who has made an outstanding contribution to the field of laboratory medicine by his research or by his service to the profession of medical technology may be recommended for honorary membership in this Society. Recommendation for honorary membership may be made by a constituent society or by the Board of Directors. A recommendation accompanied by qualifications shall be sent to the recording secretary of this Society not less than thirty days before the Annual Session. Honorary members shall be elected by a two-thirds vote of the House of Delegates. They shall be elected every third year beginning in 1954. The year when honorary members are elected each constituent society and also the Board of Directors shall be entitled to present the name of only one candidate for this honor. Honorary members shall not exceed in number one per cent of the active members of the Society in good standing at the time of their election to honorary membership. An honorary member shall be informed of his election by the recording secretary who shall send him a certificate of honorary membership signed by the president and the recording secretary. He shall enjoy all the privileges of membership except voting, holding office and serving on any Board or standing committee of this Society. He shall be exempt from payment of annual dues.
(d) Life Member. Any person who has been an active member of this Society for fifteen years and has made an outstanding contribution to this Society may be recommended for life membership by a constituent society. Election shall be by two-thirds vote of the House of Delegates. A recommendation for life membership, accompanied by the professional record of the member being recommended, shall be sent to the recording secretary of this Society not less than thirty days before the Annual Session. Not more than one person shall be presented for election to life membership by any one constituent society at any one Annual Session. A certificate of life membership signed by the president and the recording secretary shall be sent to the life member informing him of his election. A life member shall have all the rights and privileges of this Society and he shall be exempt from further payment of annual dues to this Society. A life member shall be an active member of the constituent society of the geographical area in which he is employed or resides; when a life member ceases to be an active member of a constituent society he automatically forfeits his right to life membership in this Society. Life members shall not exceed in number one per cent of the active members of the Society in good standing at the time of their election to life membership.

Section 2. Application for active or affiliate membership in this Society shall be made on a form prescribed by this Society. The transmittal to the executive secretary of a list of active and affiliate members in good standing in a constituent society, together with their annual or semi-annual dues whichever is required, shall establish them as members of this Society.

Section 3. Any member of this Society who violates the Code of Ethics or whose conduct is detrimental to this Society may be expelled by a two-thirds vote of the House of Delegates provided he has been given thirty days notice, signed by a majority of the members of the Board of Directors and sent by registered mail, stating the charges filed against him and informing him of the date of the meeting when his expulsion will be voted upon. The member so charged shall have the privilege of appearing before the House of Delegates and he may present his defense in person or through other active members of the Society of his selection.

Section 4. Any person who has been expelled from membership may be reinstated upon adoption by a two-thirds vote of the House of Delegates of his application for membership, provided the same is accompanied by annual dues to this Society plus a reinstatement fee of five dollars ($5.00).

Section 5. Each constituent society shall provide for the method of election of its members and the types and qualifications for membership in its society, except that a constituent society may grant active membership in its society only to persons who meet the requirements for active or affiliate membership in this Society; that only their active members shall have the privilege of voting and holding office in their society; and that all members of a constituent society shall abide by the Code of Ethics of this Society.

Section 6. Each constituent society shall provide for the method of election to membership in any of its branch societies and shall prescribe the types of membership in these branch societies, except that one-fourth of the members of any branch society, including its president, shall be members of this Society and that every member of a branch society shall abide by the Code of Ethics of this Society.

ARTICLE V
CODE OF ETHICS

Section 1. I will at all times work only under the direction and supervision of a pathologist or duly qualified doctor of medicine or specialist in one of the divisions of clinical pathology, such qualifications being determined on the basis of accepted medical ethics.

Section 2. I will make no diagnosis or interpretations other than those in the reports prepared by me.

Section 3. I will not advise physicians or others how to treat disease.

Section 4. I will not train students without supervision of a clinical pathologist.

Section 5. I will not engage in laboratory work independent of qualified supervision (as provided in Section 1), nor will I operate an independent laboratory.

Section 6. It is ethical to perform laboratory work on a commission basis under contract with a public health, research, or clinical laboratory when such work is done as provided in Section 1 above and when all contractual agreements are approved and signed by the director of the organization contracting for such services.

ARTICLE VI
MEETINGS

Section 1. The Society shall hold an Annual Session which shall include one or more general or scientific meetings of the members and not less than two meetings of the House of Delegates at least two of which shall be held on different days. The Annual Session shall be held at the time and place determined by the House of Delegates at any preceding Annual Session. Emergency changes concerning the time and place of an Annual Session may be made by the Board of Directors.

Section 2. The Board of Directors shall hold an annual meeting prior to the first meeting of the House of Delegates at each Annual Session.

Section 3. The Advisory Council shall hold an annual meeting prior to the first meeting of the House of Delegates at each Annual Session.

ARTICLE VII
FINANCES

Section 1. The annual dues for active and affiliate membership in this Society shall be eight dollars ($8.00) per annum.

Section 2. Dues to this Society shall be payable to the treasurer of the constituent society of the geographical area in which the member is employed or resides, or, in the event no such constituent society exists, to the executive secretary of this Society. Dues are payable on or before July 1 of each fiscal year.

Section 3. Within two weeks after the treasurer of a constituent society receives dues to this Society from a member the dues shall be transmitted to the executive secretary of this Society. Transmittal of dues to the executive secretary shall be accompanied by a duplicate classified list of paid members with their addresses and registry numbers or degree statuses.

Section 4.

(a) Any active or affiliate member who fails to pay annual dues to this Society by September 15th shall be dropped from membership. The treasurer of each constituent society shall notify the executive secretary not later than October 1st of the failure of any member to pay dues by September 15th.

(b) A person who has been dropped from membership in this Society may be reinstated at any time during the year in which he was dropped or at any later date provided he meets the requirements for membership of both his constituent society and this Society. To be reinstated in this Society he shall pay current annual dues to his constituent society with any back dues or reinstatement fee the constituent society may require, and current annual dues to this Society plus a reinstatement fee to this Society of two dollars ($2.00).

Section 5. An applicant elected to active or affiliate membership in this Society after January 1st and prior to May 1st shall pay one-half of the annual dues to this Society for the remainder of the fiscal year.

Section 6. An applicant elected to active or affiliate membership in this Society between May 1st and June 30th shall pay annual dues for one year which shall entitle him to all the privileges of his respective membership for a period up to fourteen months. He shall pay no further dues to this Society until the second fiscal year following the date of his application.

Section 7. Only new members may be granted the privilege of paying dues for one-half year. When a former member reapplies for membership his application must be accompanied by dues for one full year.

ARTICLE VIII
OFFICERS

Section 1. The officers of this Society shall be president, president-elect, recording secretary and treasurer.

(a) The president shall be the chief executive of this Society and shall preside at all meetings of the Board of Directors and of the House of Delegates. He shall be an ex-officio member of all standing and special committees. He shall set up such standing committees as are provided for in the By-Laws. He shall appoint, with the approval of the Board of Directors, such special committees as are needed.

(b) The president-elect shall, during his term of office, familiarize himself with the duties which shall devolve upon him as president. In the event of the death, resignation or total disability of the president, the president-elect shall perform the duties of, and have the same authority as, the president.

(c) (1) the recording secretary shall act as secretary at all meetings of the Board of Directors and of the House of Delegates.

(2) At the first meeting of the House of Delegates at each Annual Session the recording secretary shall read the minutes of the meetings of the House of Delegates of the previous Annual Session and the minutes of the meetings of the Board of Directors held since the first meeting of the House of Delegates of the last Annual Session.

(3) He shall keep in permanent form in his possession a record of all minutes taken at all meetings at which he serves, and shall provide each of the members of the Board of Directors and the executive secretary with a copy of these minutes within thirty days after the close of the Annual Session at which the minutes were taken.

(4) He shall carry on such correspondence as shall be authorized by the House of Delegates and Board of Directors of this Society.

(5) To aid the recording secretary in the keeping of an accurate record of the annual meetings of the House of Delegates the Society shall employ an expert stenographer to record all the proceedings of the meetings of the House of Delegates. Such record shall be delivered to the president of this Society by the stenographer within thirty days after the close of an Annual Session. The president shall within thirty days after receipt of the record transmit the same to the executive secretary to be filed in the executive office.

(6) At the end of his term of office the recording secretary shall submit all records, including the minute book, to his successor.

(7) In the event of the simultaneous inability of both the president and the president-elect to perform the duties of president, the recording secretary shall perform the duties of, and have the same authority as, the president.

(d) The treasurer shall receive, record and have charge of all funds of this Society. He shall deposit these funds in a bank designated by the Board of Directors and pay all bills approved by the Board of Directors or the House of Delegates upon receipt of vouchers signed by the chairman of the Board. He shall be bonded at the expense of the Society. He shall, within the month previous to the Annual Session, have his accounts audited by a public accountant at the expense of the Society. He shall submit to the House of Delegates at each Annual Session an itemized statement of all receipts and expenditures of the Society for the year just ended. At the end of his term of office he shall deliver his current records and all cash on hand to his successor. All other records in his possession shall be transmitted to the executive secretary to be filed in the executive office.

Section 2. Eligibilty for Office. Any person who is, and for at least three years prior to his election or appointment has been, an active or life member of this Society shall be eligible to hold office.

Section 3. Method of Election.

(a) All officers of this Society shall be elected by the House of Delegates at the Annual Session of this Society.

(b) An official list of candidates for each office shall be presented to the House of Delegates by the nominating committee, the list of candidates and their qualifications having been sent to each member of this Society at least sixty days before the Annual Session. Nominations may be made from the floor.

(c) The election of officers by the House of Delegates shall be conducted by an elections committee consisting of five members of the House of Delegates appointed by the chair.

(d) Election shall be by ballot and a majority of votes cast elects. If none of the candidates for a certain office receives a majority vote on the first ballot the candidate with the lowest number shall be eliminated and balloting shall continue until a candidate receives a majority of the votes cast. In the event of a tie, election shall be determined by lot.

Section 4. Term of Office.

(a) The president-elect shall be elected for a term of one year and, except in the event of a vacancy in the office of president by death, resignation or total disability, he shall serve for one year as president-elect and then automatically succeed to the office of president.

(b) The recording secretary shall be elected for a term of one year. He may be reelected. He shall not serve in this office for more than two years.

(c) The treasurer shall be elected for a term of three years. He may be reelected. He shall not serve in this office for more than six years.

(d) The term of office of any officer of this Society elected at an Annual Session shall begin with the sine die adjournment of the House of Delegates at such Annual Session.

Section 5. Filling of Vacancies.

(a) In the event of the death, resignation or total disability of the president, the president-elect shall become the president of this Society for the remainder of the year of the president's term of office and for the following year, and the Board of Directors shall elect by majority vote a president-elect to serve for the remainder of the year in which the vacancy occurs. At the next Annual Session following the occurrence of the vacancy in the office of president, the House of Delegates shall elect a president-elect for a term of one year.

(b) In the event of the simultaneous removal during their term of office of both the president and the president-elect by death, resignation or total disability the recording secretary shall become the president of this Society for the remainder of their term of office. The Board of Directors shall by majority vote elect a president-elect from the slate of nominees for that office from the previous election and a recording secretary from the slate of nominees for that office from the previous election, both of whom shall be elected to serve for the remainder of the year in which the vacancies occur.

(c) In the event of removal from office of the recording secretary by death, resignation or total disability the Board of Directors shall by majority vote elect a recording secretary from the slate of nominees for that office from the previous election to serve for the remainder of the year in which the vacancy occurs.

(d) In the event of removal from office of the treasurer by death, resignation or total disability the Board of Directors shall by majority vote elect a treasurer from the slate of nominees for that office from the previous election to serve for the remainder of the term of office for which his predecessor was elected.

ARTICLE IX
EXECUTIVE SECRETARY

Section 1. The executive secretary shall be elected by the Board of Directors, shall serve under a written contract, and, subject to the action of the House of Delegates, shall be under the supervision and control of the Board. He shall, under the direction and control of the House of Delegates and the Board of Directors, maintain a central office for the Society in which the records, properties, bonds and legal papers of the Society shall be preserved in properly maintained files. He shall do the administrative work necessary to the operation of the Society and shall be the business manager of the official publication.

Section 2. The executive secretary shall contract for technical exhibits for the Annual Session of the Society and, working with the local chairman of exhibits, shall arrange for all technical exhibits. All receipts from technical exhibits shall go into the treasury of this Society.

ARTICLE X
BOARD OF DIRECTORS

Section 1. The Board of Directors shall consist of the president, the president-elect, the recording secretary, the treasurer, the immediate past-president and six members of this Society elected by the House of Delegates.

Section 2. The Board of Directors shall represent the Society when the House of Delegates is not in session and only when urgent business necessitates immediate action that cannot wait until the House of Delegates reconvenes. In no case, except in an emergency, shall the Board of Directors transact business that vitally affects the interests of this Society and in no case shall the Board of Directors take any action which binds the Society beyond the next ensuing Annual Session, except as specific authority for such action has been previously granted by the House of Delegates.

Section 3. Any person who is, and for at least three years prior to election or appointment has been, an active or life member of this Society shall be eligible to serve on the Board of Directors.

Section 4. Method of Election.

(a) Two members of this Society shall be elected annually to the Board of Directors by the House of Delegates each to serve for a term of three years.

(b) The nominating committee shall prepare two slates of nominees, one for each vacancy on the Board. Nominations may be made from the floor.

(c) Election shall be conducted by the elections committee.

(d) Election shall be by ballot, voting continuing until a candidate receives a majority vote.

Section 5. The term of office of any member of the Board of Directors elected at an Annual Session shall begin with the sine die adjournment of the House of Delegates at such Annual Session.

Section 6. The president of this Society shall serve as chairman of the Board of Directors and the recording secretary shall serve as secretary of the Board.

Section 7. The president or president-elect and five other members of the Board shall constitute a quorum.

Section 8. The annual meeting of the Board of Directors shall be held within two weeks prior to the first meeting of the House of Delegates at each Annual Session, the time and place of such meeting being designated by the chairman.

Section 9. In the event of removal by death, resignation or total disability of a member of the Board of Directors who is not an officer of this Society, the president of this Society shall appoint one of the candidates from the slate of nominees for members of the Board of Directors from the preceding election to serve until the next Annual Session. At the next Annual Session the vacancy shall be filled by election by the House of Delegates.

ARTICLE XI
HOUSE OF DELEGATES

Section 1. The membership of the House of Delegates shall consist of the members of the Board of Directors, the members of the Advisory Council and the delegates elected by their constituent societies or appointed in accordance with the provisions of these By-Laws.

Section 2. Each constituent society shall elect, up to ten delegates, one delegate and one alternate for each 25 active, life, and affiliate members, or fraction thereof, of the constituent society. When the membership of a consituent society exceeds 250, such society shall elect an additional delegate and an additional alternate for each additional 50 active, life, and affiliate members, or fraction thereof, above 250.

Section 3. The number of delegates to which any constituent society is entitled shall be determined by the number of life members from that constituent society in this Society plus the number of active and affiliate members who have paid current dues to, and are in good standing in, this Society. A member who has transferred from one constituent society to another shall be counted as a member of the geographical area in which he holds his membership thirty days prior to the opening of the Annual Session.

Section 4. The secretary of each constituent society shall send to the executive secretary of this Society at least two weeks before the opening of the Annual Session, and earlier if possible, a list of delegates and alternates from his Society with their addresses and registry numbers or degree statuses.

Section 5. If the combined number of delegates and alternates present at any Annual Session from any one constituent society is less than the quota of delegates for that society, the president of that constituent society, or in his absence the other officers of that society, shall fill the quota from eligible active and life members present at the Annual Session. When necessary the president of this Society shall name these delegates.

Section 6. Each delegate and each alternate shall present his credentials to the chairman of the convention credentials committee when he registers at the Annual Session.

Section 7. The members of the Board of Directors and of the Advisory Council and the officers of this Society shall not be included in the list of elected or appointed delegates from a constituent society to the House of Delegates.

Section 8. Delegates and alternates shall serve from the time of the first meeting of the House of Delegates at the Annual Session for which they were elected as delegates and alternates to the first meeting of the House of Delegates at the next ensuing Annual Session.

Section 9. A quorum of the House of Delegates shall be a majority of its accredited members present at the Annual Session.

Section 10. Any member in good standing in this Society is eligible to attend the annual meetings of the House of Delegates and to participate in discussion but the privilege of the floor shall be granted to a non-delegate only by consent of the House. Only members of the House of Delegates can vote.

ARTICLE XII
ADVISORY COUNCIL

Section 1. The Advisory Council of this Society shall consist of those persons who are the presidents of the constituent societies of this Society and those persons who are the members of the Board of Directors of this Society as of the day immediately following the sine die adjournment of the House of Delegates at any Annual Session. These same persons shall remain on the Advisory Council until the sine die adjournment of the House of Delegates at the next ensuing Annual Session even though in that period the presidents of constituent societies on the Council have become the past presidents of their respective constituent societies. With the exception of the members of the Board of Directors, it shall be the privilege of a member of the Council to appoint any other active or life member of his constituent society as his alternate when he is unable to be present at a meeting of the Council. Only one active or life member from a constituent society shall be allowed certification as a member of the Advisory Council at any one Annual Session of this Society.

Section 2. The purpose of the Advisory Council shall be to consider matters of importance pertaining to the interest and welfare of the members of this Society and of the members of the profession of medical technology in general. The Advisory Council shall have no power to act for the Society. It may make recommendations to the House of Delegates. By means of a report made by the chairman or other duly authorized member of the Council to the House of Delegates it may bring matters of importance to the attention of the House of Delegates.

Section 3. Officers of the Advisory Council shall consist of a chairman, a vice-chairman, a recording secretary, an assistant recording secretary and a corresponding secretary.

(a) Officers of the Advisory Council shall be elected at a meeting following soon after the sine die adjournment of the House of Delegates at each Annual Session.

(b) No member of the Board of Directors shall hold office in the Advisory Council.

(c) The president of this Society shall call a meeting of the Advisory Council soon after the sine die adjournment of the House of Delegates for the purpose of organizing the Advisory Council. He shall act as chairman pro tem of this meeting. He shall read, or distribute in writing, to the members of the Council a list of the persons elegible for office. Nominations shall be made from the floor. Election shall follow. A majority vote of all members of the Advisory Council present at that meeting of the Council shall elect.

(d) A quorum of the Advisory Council shall be a majority of its members.

(e) Officers shall take office immediately after their election and they shall remain in office until the sine die adjournment of the House of Delegates at the next ensuing Annual Session.

Section 4. The duties of the officers of the Advisory Council shall be as follows:

(a) The chairman shall preside at all meetings of the Council. He shall provide for the preparation and presentation of reports and recommendations from the Advisory Council to the House of Delegates.

(b) The vice-chairman shall in the absence of the chairman assume the duties of the chairman.

(c) The recording secretary shall take the minutes of the meetings of the Advisory Council and shall keep a permanent record of these minutes. He shall, within thirty days after the close of the Annual Session, prepare and distribute to the members of the Advisory Council a copy of the minutes of the meetings held by the Council at the time of the Annual Session just closed.

(d) The assistant recording secretary shall in the absence of the recording secretary assume the duties of the recording secretary.

(e) The corresponding secretary shall carry on the correspondence for the Advisory Council.

Section 5.

(a) The annual meeting of the Advisory Council shall be held just prior to the first meeting of the House of Delegates at each Annual Session. The chairman of the Council in conjunction with the president of this Society shall designate the time and place of such meeting.

(b) Any member in good standing in this Society shall be eligible to attend the meetings of the Advisory Council and to participate in discussion, but the privilege of the floor shall be granted to a non-member of the Council only by consent of the Council. Only members of the Council can vote.

(c) Each constituent society shall be entitled to one and only one voting member at any meeting of the Council.

Section 6. An appropriation of money for the activities of the Council can be made only by a two-thirds vote of the House of Delegates.

ARTICLE XIII

REPRESENTATION ON THE BOARD OF REGISTRY

Section 1. The president, the president-elect and the immediate past-president of this Society shall represent the Society as members of the Board of Registry of Medical Technologists of the American Society of Clinical Pathologists.

ARTICLE XIV

REPRESENTATION ON THE BOARD OF APPROVED SCHOOLS

Section 1. Three representatives of this Society shall be elected by the House of Delegates to serve on the Board of Approved Schools of the American Society of Clinical Pathologists. Nominations shall be made by the nominating committee and election shall be by ballot. A majority of the votes cast elects.

Section 2. Persons who are, and for at least five years prior to election or appointment have been, active or life members in good standing of this Society shall be eligible for election to the Board of Approved Schools provided, however, that such persons shall hold academic degrees at least at a baccalaureate level and have had at least three years of teaching experience within the current decade in a clinical laboratory or in a school of medical technology approved by the Council on Medical Education and Hospitals of the American Medical Association and that they shall have held office either in this Society or in one of its constituent societies or have held membership on the Board of Directors of this Society or on a comparable Board of a constituent society.

Section 3. A representative to the Board of Approved Schools shall be elected to this position for a term of three years. One representative shall be elected at each Annual Session. In 1953 three representatives shall be elected, one to serve for one year, one for two years and one for three yars.

Section 4. A representative to this Board shall not serve for more than two terms or more than six years.

Section 5. In the event of the death, resignation or total disability of a representative to the Board of Approved Schools the Board of Directors shall fill the unexpired term by appointment.

ARTICLE XV
ELECTIONS COMMITTEE

All elections by the House of Delegates shall be conducted by an Elections Committee consisting of five delegates appointed by the chair. Each member of the Elections Committee shall receive and carry out the list of instructions previously prepared by the chair in accordance with the parliamentary authority of the Society.

ARTICLE XVI
SPECIAL COMMITTEES

Section 1. Special committees may be appointed at any time by the president on authority given him by the House of Delegates or the Board of Directors.

Section 2. The president shall appoint the necessary personnel for committees that shall arrange for the Annual Session of this Society.

ARTICLE XVII
STANDING COMMITTEES

Section 1. There shall be ten standing committees of six members each of such geographical distribution as shall be conducive to effective work. These shall be as follows: Constitution and By-Laws; Education; Legislation; Membership; Nominations; Public Relations; Research; Service Fund and Finance; Standards and Studies; Vocational Guidance and Recruitment.

Section 2. At the beginning of his term of office the president of this Society shall appoint, each for a term of three years, two members to each of the standing committees to succeed the two members whose term ended with the sine die adjournment of the House of Delegates at that Annual Session. The president shall appoint a chairman for each standing committee for a term of one year. A member may not serve for more than six consecutive years on the same standing committee. Active, honorary and life members may be appointed to membership on standing committees.

Section 3. The duties of the standing committees shall be as follows:

(a) (1) The Constitution and By-Laws Committee shall examine all proposed amendments to the By-Laws of this Society and shall make such changes as are necessary to put them in proper form without effecting any changes in their meaning. Proposed amendments to the By-Laws of this Society may be submitted by an active or life member of this Society or by a constituent society. After the necessary changes have been made all such proposed amendments shall be distributed to each active and life member of this Society not less than sixty days prior to the next Annual Session of this Society.

(2) The Constitution and By-Laws Committee shall examine the proposed Constitution, or Articles of Incorporation, and By-Laws of any group petitioning for a charter from this Society and shall bring a report and a recommendation concerning the chartering of such group to the House of Delegates at the next ensuing Annual Session of this Society.

(3) The Constitution and By-Laws Committee shall examine all proposed amendments to the Constitution and By-Laws of a constituent society and shall submit the same to the chairman of the Board of Directors with an opinion. If both the Committee and the Board of Directors approve the proposed amendments to the By-Laws of the constituent society, the proposed amendments shall be presented to the membership of the constituent society to be voted on in the manner prescribed by the By-Laws of said society. The constituent society shall report the adoption of any amendments to its Constitution and By-Laws to the chairman of the Constitution and By-Laws Committee of this Society within two weeks after the adoption of such By-Laws.

(b) The Education Committee shall develop teaching aids, encourage the establishment of laboratory libraries, prepare and make available to members and to constituent societies current lists of visual aids, study the curricula of approved schools of medical technology and aid graduate and undergraduate students of approved schools of medical technology in every way possible.

(c) The Legislation Committee shall assume the duties assigned to it by the House of Delegates and Board of Directors.

(d) The Membership Committee shall plan ways and means of cooperating with the membership committees of constituent societies with the aim of increasing the membership of this Society and of the constituent societies.

(e) The Nominations Committee shall publish in the September-October issue of the official publication of this Society the names of Officers, Directors and Representatives for the Board of Approved Schools whose term of office expires at the next Annual Session. It shall solicit from the members of this Society names of qualified candidates and their qualifications for offices and other respective positions to be filled by the House of Delegates at the next Annual Session. A list of candidates shall be distributed to each active and life member of this Society not less than sixty days before the date of the next Annual Session. Only the names of persons who have consented to serve if elected shall be listed.

(f) The Public Relations Committee shall, by dispensing information and promoting public understanding of the profession of medical technology, act as a liaison group between this Society and other professional or lay groups.

(g) The Research Committee shall direct the research activities of this Society.

(h) The Service Fund and Finance Committee shall stimulate fund raising for service to the undergraduate and graduate medical technologist and shall comply in so far as possible with the suggestions of the Board of Directors and House of Delegates regarding policies and practices relative to the use of the fund. Disbursements from the fund shall be made by voucher signed by the chairman of the Board of Directors. A separate financial account shall be kept for the fund. This shall be audited as provided for the other financial accounts of the Society. At each Annual Session of this Society an itemized statement of all receipts and expenditures to the fund during the past year shall be presented to the House of Delegates. The Service Fund and Finance Committee shall also study ways and means of meeting allotments established in the budget and shall prepare a budget for the coming year to be presented to the House of Delegates at each Annual Session.

(i) The Standards and Studies Committee shall study medical technology ethics, economics, standards and other kindred problems. This Committee shall inform the members of this Society of the activities of other professional medical technology organizations. It shall promote better understanding of professional medical technologists by the public so that the public may discriminate between adequately and inadequately trained members of our profession.

(j) The Vocational Guidance and Recruitment Committee shall have the responsibility of recruiting students into the field of medical technology.

ARTICLE XVIII
FISCAL YEAR

The fiscal year shall be from July 1 to June 30, inclusive.

ARTICLE XIX
OFFICIAL PUBLICATION

Section 1. The official publication of this Society shall be THE AMERICAN JOURNAL OF MEDICAL TECHNOLOGY, the name of which is patented and the contents of which shall be copyrighted.

Section 2. The official publication shall be made available to all active and affiliate members of this Society who have paid their dues and are in good standing, and to all honorary and life members.

Section 3. The Editor of the official publication shall be elected by the Board of Directors and shall serve under written contract.

Section 4. The Editorial Board shall consist of the Editor, two or more Advisory Editors and two or more Associate Editors. The Advisory and Associate Editors shall be appointed annually by the Editor with the approval of the Board of Directors.

ARTICLE XX

The executive office of the Society shall be located at Houston, Texas.

ARTICLE XXI
PARLIAMENTARY AUTHORITY

Robert's Rules of Order Revised shall govern the business proceedings of this Society except when otherwise specified in these By-Laws.

ARTICLE XXII
AMENDMENTS

Section 1. The By-Laws of this Society may be amended as follows:

(a) Any proposed amendment to these By-Laws recommended by either a constituent society or by an active or life member of this Society shall be submitted in writing to the chairman of the Constitution and By-Laws Committee not later than December 1st. Six copies of the proposed amendment shall be submitted. The Constitution and By-Laws Committee shall make such changes as may be necessary to provide proper form without altering the meaning of the amendment. After such changes have been made the proposed amendment shall be distributed to each active and life member of this Society not less than sixty (60) days prior to the opening of the next Annual Session, at which Session the proposed amendment shall be presented to the House of Delegates for vote.

(b) Amendments to these By-Laws shall be adopted by a two-thirds vote of the House of Delegates, except that a proposed amendment to Article XX of these By-Laws shall be approved by a two-thirds vote of the Board of Directors before it may be submitted to the House of Delegates for vote.

Section 2. A complete revision of these By-Laws shall be presented to a meeting of the House of Delegates at an Annual Session only upon two-thirds vote of the House of Delegates at one Annual Session ordering a revision of the By-Laws for presentation to a meeting of the House of Delegates at the next ensuing Annual Session. A two-thirds vote of the House of Delegates shall be necessary for adoption of a revision of these By-Laws.